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Brucellosis and Q-fever seroprevalences of nomadic pastoralists and their livestock in Chad

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Abstract

As a part of a research-and-action partnership between public health and veterinary medicine, the relationships between the seroprevalences of brucellosis and Q-fever in humans and livestock were evaluated in three nomadic communities of Chad (Fulani cattle breeders, and Arab camel and cattle breeders). Nomad camps were visited between April 1999 and April 2000. A total of 860 human and 1637 animal sera were tested for antibodies against *Brucella* spp., and 368 human and 613 animal sera for *Coxiella burnetii*. The same indirect ELISA was used for livestock and human sera, and the test characteristics for its use on human sera were evaluated. Twenty-eight people were seropositive for brucellosis (seroprevalence 3.8%). Brucella seroprevalence was higher in cattle (7%) than other livestock, and brucellosis seropositivity was a significant factor for abortion in cattle (OR = 2.8). No correlation was found between human brucellosis serostatus and camp proportions of seropositive animals.

Q-fever-seropositive blood samples were taken from 11 Arab camel and 4 Arab cattle breeders (seroprevalence 1%). Being a camel breeder was associated with Q-fever seropositivity in humans (OR = 9). Camels had the highest Q-fever seroprevalence (80%) among livestock species.

Although there was high-risk human behaviour for the acquisition of brucellosis and Q-fever from livestock through raw-milk consumption (98%) and contact with placentas of livestock (62%), we concluded that seroprevalences in humans were relatively low (likely due to limited active foci in livestock).

Keywords: Zoonoses; Pastoralism; Chad; Brucellosis; Q-fever

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1. Introduction

In the Sahelian zone, an estimated 16% of the 35 millions population are mobile pastoralists who depend on their livestock for subsistence (Bonfiglioli and Watson, 1992). About 10% of the total population of Chad are nomads, but studies on morbidity and mortality of nomadic pastoralists are few and out-dated. The highest density of nomadic pastoralists is found in the dry Sahel zone, south of the Sahara. The zoonoses brucellosis and Q-fever might play an important role in the disease burden of these populations, because pastoralists live in close contact with their animals.

"Classical" zoonoses such as bovine tuberculosis, brucellosis, anthrax, and rabies are still widespread in Africa, with mostly unknown human and animal welfare costs (Meslin et al., 2000). Brucellosis is considered by the Food and Agriculture Organization (FAO), the World Health Organization (WHO) and the "Office International des Epizooties" (OIE) as one of the most-widespread zoonoses in the world. Brucellosis in humans (due to *Brucella melitensis* or *B. abortus*) causes an acute febrile disease with undulant fever, which can progress to a more-chronic form; there also can be serious complications affecting the musculo-skeletal, cardiovascular and central nervous systems. Animals are the almost-exclusive source of infection for people. *B. melitensis* shows a host preference for goats and sheep, and *B. abortus* for cattle (Blood and Radotstits, 1989). In sub-Saharan Africa, the highest incidences of brucellosis are found in pastoral production systems. McDermott and Arimi (2002) stated that in sub-Saharan Africa, the epidemiology of brucellosis in humans and livestock (as well as cost-effective prevention measures) are not well understood and available data are limited.

One-fourth of nomadic pastoralists of the Malian Gourma had antibodies against the bacterial agent of brucellosis; of the five pastoralist communities tested, the seroprevalence was lower than 25% only in the group which had lost all its livestock in previous years (Chabasse et al., 1983). Another study compared these results with sedentary cultivators in three other settings in Mali. Seroprevalences ranged from 0 to 4% among cultivators (Tasei et al., 1982). These two studies did not attempt to associate the seroprevalences in animals to these in humans. Gidel et al. (1974) sampled sera from livestock and people in the same villages of different zones and ethnic groups (livestock breeders and crop farmers) in Côte d'Ivoire, Burkina Faso and Niger. However, those authors found no relationship in seropositivity between people and livestock. Antibodies against *Brucella* spp. were found repeatedly in sera of cattle and small ruminants in Chad—with large differences between different regions, authors and time periods as generally seen in sub-Saharan Africa (McDermott and Arimi, 2002). Seroprevalences for cattle ranged from 3 to 30% (Domenech et al., 1982; ME, 2000). For the human population, seroprevalence was 14% for slaughterhouse workers—but 0% in a group of urban blood donors (Massenet et al., 1993). Lefèvre et al. (1970) isolated *Brucella* strains (mainly *B. melitensis*, but also *B. abortus*) from 12 patients suspected of brucellosis in Chad.

The rickettsial disease Q-fever (caused by *Coxiella burnetii*) can be transmitted by ticks to domestic animals. Yet, the transmission pathways from livestock to humans are very similar to those of brucellosis, including the consumption of non-pasteurised dairy products, the contact with diseased animals and carcasses, or with products of livestock births and abortions (infection is acquired most often by the respiratory and conjunctival route through

aerosols or contaminated dust). Antibodies to Q-fever were found in 3.5% of sera collected in N'Djaména, Chad (Maurice and Gidel, 1968). Those authors concluded that coxiellosis may be responsible for a number of undefined cases of fever. During the same study in Chad, positive microagglutination reactions were observed for cattle, sheep, goats and camels. Distinctively higher seroprevalences (35–75%) were found in humans in a study conducted by Giroud et al. (1951) in southern Chad, but the study population had close contact with livestock (breeders, butchers and meat sellers).

We established an intersectoral collaboration between public health and livestock production to evaluate the relationship between the seroprevalences of brucellosis and Q-fever in humans and animals of same nomadic camps, and to identify possible sources of exposure of pastoralists to these two zoonoses.

2. Materials and methods

A research team consisting of veterinarians and physicians collaborated with the Ministry of Health in Chad and the National Veterinary Laboratory of Chad (Laboratoire de Recherches Vétérinaires et Zootechniques de Farcha). Furthermore, in the context of a broader research programme on the health of nomadic people in Chad by the Swiss Tropical Institute, the group collaborated with an anthropologist and a geographer. Quality control of the brucellosis and Q-fever serology was conducted at the Institute of Veterinary Bacteriology of the University of Berne, Switzerland and the Valais Central Laboratory, Sion, Switzerland, respectively.

2.1. Sampling and data collection

The study took place in the Chadian provinces Chari-Baguirmi and Kanem. Nomadic Fulani cattle breeders and Arab camel or cattle breeders were included in the study to consider two different types of livestock breeding. The following multistage selection procedure of nomadic camps was adopted before the actual sampling: based on established contacts by the geographer and his preliminary knowledge of transhumance routes, two Fulani and two Arab representatives of different nomadic sub-groups in the pre-defined study zone were contacted. Each representative was asked to identify 10 concentration zones of nomadic camps (usually defined by the name of the main village in the vicinity). Camp elders (boulamas) were contacted at markets, weddings, and circumcision ceremonies or by following a herd to the camp in five (selected with two dice) out of the 10 zones. In total, approximately 100 boulamas were asked for participation in the study after they were informed about the objectives and the sampling procedure. Half of these boulamas did not want to participate (the main reason given was the blood sampling of the people). Out of the list with boulamas willing to participate, 15 camps per ethnic group were selected with random numbers. In addition, the camp of one important leader per ethnic group needed to be included to ensure acceptance among pastoralists.

Sixteen Fulani and 15 Arab camps were visited during a first sampling in May/June 1999 (hot dry season) with the help of guides with a good knowledge of the approximate geographical distribution of camps. The sample size of 30 camps to visit was feasible.

Twenty-seven of these camps were visited again up to 400 km further north or east during a second sampling in October/November 1999, after the annual rainfalls. During a third sampling in March/April 2000, six Fulani and four Arab camps were visited for a third time. In addition, 12 Fulani and 11 Arab camps were newly included in the study. A unique identification number was allocated for each visited camp, which was retained for all samplings.

In each visited camp, five men (≥15 years old), five women (≥15 years old) and five children (boys or girls, 1 month to 14 years old) were selected with random-number cross-tables prepared for different group sizes of men, women, and children. The selection of numbers, which were handed out to all individuals at arrival, was made with two dice. The median numbers of tents (representing approximately one family), men, women and children per camp were 7, 7, 10 and 25, respectively, for Fulani; and 8, 10, 10 and 23 for Arabs. After a complete physical examination (recording of, e.g. fever, splenomegaly, hepatomegaly, pale conjunctiva), a pre-tested structured questionnaire including typical signs of brucellosis (such as nature of fever and headache, muscle and joint pain, tiredness) as well as factors either known or thought to influence the transmission from livestock to humans of brucellosis and Q-fever was completed with participants. In addition, reported symptoms and duration of illness were recorded. Venous blood was taken with 5 ml Vacutainer® tubes with informed consent of each participant or of young children's mothers. Whole blood was centrifuged with a mobile centrifuge for 10 min at 5000 rpm. Serum was transported on ice in 2 ml tubes to the laboratory. Each tube was labelled with a code including an individual sampling number, and information on age-class, sex, ethnic group, and camp identification. The physician treated sick people in the camps at no charge. People who could not be treated immediately were referred to local dispensaries. Children <5 years old were vaccinated against measles, BCG, and yellow fever during the second and third sampling.

Animals of different owners (usually family members) normally were herded separately during the day—but animals of one camp intermixed during the night (although small-ruminants and camels or cattle have separate rest areas). Animals were sampled early in the morning or before sunset on the camp site. Blood specimens from 10 cattle or 10 camels (*Camelus dromedarius*), as well as from five sheep and five goats—almost exclusively females in lactation for all species—were obtained by venipuncture with 5 or 10 ml Vacutainer® tubes. Milk samples from the same females were collected in sterile 10 ml tubes. All tubes were labelled with a code including an individual sampling number, and information on species, and camp identification. A semi-quantitative assessment of tick infestation on animals was done (no ticks, ticks <10, 10 ≤ ticks < 100, ticks ≥ 100 per animal). The breed, age, name, number of births, and number of abortions were registered for each animal on the basis of information from the owner. The veterinarian treated sick animals.

2.2. Serological tests

2.2.1. Brucellosis serology

All human and livestock sera were subjected to the Rose-Bengal plate-agglutination test (RBT); 30 μl of serum and 30 μl of antigen (*B. abortus* strain 99, Sanofi Diagnostics

Pasteur, Marnes-la-Coquette, France) were mixed and rotated on a glass plate for 4 min. Agglutination values were recorded as negative, +, ++, +++ and ++++. Sera with values ≥++ (all with time-to-reaction <2 min) and <++ were classified as positive and negative, respectively. The RBT is a simple and inexpensive test to detect antibodies against *Brucella* spp. in serum of many species. However, sensitivity and specificity vary in different settings and depending on investigators (Maichomo et al., 1998; Ostanello et al., 1999). Possible non-specific reactions due to cross-reactions (e.g. with *Yersinia* spp.) which can cause false-positive *Brucella* RBT results also must be considered (Chart et al., 1992; OIE, 1996).

A commercially available indirect enzyme-linked immunosorbend assay (ELISA) (CHEKIT®-Brucellose, Dr. Bommeli AG, Liebefeld-Bern, Switzerland) also was used because it has a better reproducibility of results compared to the RBT, but is technically simpler to perform than the complement-fixation test (CFT). This assay uses microtiter plates with wells precoated with a lipopolysaccharide–phenol extract of the *B. abortus* 99 Weybridge strain and, as conjugate, a monoclonal anti-ruminant-IgG (also reacting with IgG of different animal species, including human). The protocol of the ELISA manufacturer was followed with the exception of a shorter time to reaction of the chromogen (stopped after 5–10 min instead of 25 min) because of higher room temperatures in Chad compared to Switzerland. The optical densities (OD) of all samples were tested in duplicate to obtain the mean OD, and doubtful duplicates were re-assayed. Results were expressed as the percentage of the ratio between the corrected sample OD and positive control OD (S/P-ratio) and was calculated as follows:

$$\frac{S}{P} = \frac{\text{mean OD}_{\text{sample}} - \text{mean OD}_{\text{negative control}}}{\text{mean OD}_{\text{positive control}} - \text{mean OD}_{\text{negative control}}} \times 100\%$$

RBT-positive camel sera from another study from a herd with signs of clinical brucellosis were tested. The ELISA was positive for the seven RBT-positive camel sera and was negative for 37 RBT-negative samples. The S/P-ratio-value ≥100% (recommended by the manufacturer) was used for classification of brucellosis seropositive livestock sera. The specificity and sensitivity of this test in detecting antibodies against *Brucella* spp. relative to the complement-fixation test are 99.9 and 96.8%, respectively (Behring Diagnositic, 1994). The specificity and sensitivity to detect antibodies against *B. melitensis* are 82 and 93% relative to the CFT, and these test characteristics were used for small-ruminant sera (predominance of *B. melitensis* infections) (Bommeli, 2000).

Two human reference brucellosis sera and two RBT-positive field human sera showed a good correlation ($r^2 = 0.84$) between optical densities (OD) obtained with the monoclonal anti-ruminant-IgG-peroxidase-conjugate and anti-human-IgG-peroxidase-conjugate (Sigma) diluted 1:10,000 at 5, 10, 20, and 30 min of reaction with the ELISA chromogen. Human sera were evaluated further, due to lower affinity to anti-*ruminant*-conjugate and due to RBT-positive but ELISA-negative results which could not alone be explained by "a too high cutoff value" for the human sera. Twelve RBT-positive human sera (out of a total of 19 RBT-positive) and 26 RBT-negative human sera were selected with computerised random numbers and tested with the CFT following the protocol of the OIE manual (1996). Receiver-operating characteristic (ROC) analyses (reference variable consisting of positive [RBT- or CFT-positive] and negative [RBT- and CFT-negative] samples) were used

to determine an appropriate positive threshold (by intervals of samples). The cutoff should yield a high specificity, but consider a putative low seroprevalence to minimise false classification of human sera. The ROC plot was moderately accurate (AUC = 0.88) (Greiner et al., 2000). The selected cutoff (S/P-ratio value of 75%) also was examined by visual observation of frequency distributions of serum samples. Specificity and sensitivity were 100 and 60%.

All RBT-positive sera were tested with the ELISA using the same protocol as described above, but now with an anti-human-IgM-peroxidase-conjugate (Sigma^{TM}) diluted 1:12,000. The ROC-curve analyses performed as described above indicated an appropriate cutoff at the S/P-ratio value of 30% (AUC = 0.88).

2.2.2. Q-fever serology

The indirect ELISA (CHEKIT$^{®}$-Q-fever, Dr. Bommeli AG, Liebefeld-Bern, Switzerland) was used to assay for antibodies against *C. burnetii* in blood sera of livestock and humans (collected during the first sampling) according to the manufacturer's instructions. Results were recorded as positive when S/P-ratio-values were ≥40%. At this threshold-value (recommended by the manufacturer), a good visual cutoff was seen for all species. Specificity and sensitivity of this test are 100 and 92% relative to the CFT and indirect immunofluorescence test (IFAT) (Thiele et al., personal communication cited in Bommeli, 1997). Thirteen out of 15 Q-fever ELISA-positive results of human sera were also positive with the IFAT using phase-I and phase-II *C. burnetii* antigens (Valais Central Laboratory, Sion, Switzerland).

2.3. Culture of milk for brucellosis

A sample of 10 ml of aseptically collected milk was stored at 4 °C overnight. A selective supplement for the isolation of *Brucella* species (OXOID^{TM}) was added to Columbia agar containing ethyl violet. Supernatant milk cream was spread on the agar and incubated at 37 °C in air supplemented with 10% CO_2 for at least 5 days. Presumptive identification of *Brucella* spp. was based on purple coloration of colonies, colony morphology, Gram stain, and agglutination with monospecific antiserum. Further testing included CO_2 requirement, H_2S production and PCR method to determine genus (Herman and De Ridder, 1992). Definitive *Brucella* spp. identification was done at the German National Veterinary Medical Reference Laboratory for Brucellosis (BGVV, Berlin-Marienfelde).

2.4. Data analyses

Blood samples and questionnaire data of same individuals (identified by their name and age) having been examined a second ($n = 47$) or third time ($n = 4$) in a subsequent sampling were withdrawn from analyses to avoid repeated sampling. Among these 51 samples, none was brucellosis seropositive. We used Intercooled STATA 7.0 for Windows (Stata Corporation, Texas, USA) for data analyses. Logistic-regression models with random-effect (RE) on the camp level (xtlogit procedure) were used to estimate apparent seroprevalences (pooled from the three samplings). The apparent seroprevelances were converted to estimated true seroprevalences using the formula developed by Rogan and Gladen (1978) and confidence intervals were calculated using the formula of Abel (1993).

Factors possibly associated with seropositivity in humans were evaluated with logistic-regression models (with RE at the camp level) using backward stepwise selection and a removal level for covariates at $P = 0.10$ based on the likelihood-ratio test (LRT). Separate models were established for brucellosis and Q-fever with the same variables. Variables were sampling number, group (Fulani, Arab camel breeder, Arab cattle breeder), age (categorised as 5–14, 15–44, and ≥45), sex, consumption of raw milk, contact with livestock placentas and/or abortion material, performance of obstetric work for livestock (for the last four variables, detailed information on frequency and livestock species was recorded for further analyses). Meaningful interactions (sex and age category, ethnic group and behavioural factors) were tested. The RE logistic-regression procedure also was used to evaluate factors influencing livestock seropositivity within each species (sampling, age, parity, breed (for cattle), and ethnic group of owner). The ordinal data on tick infestation was dichotomised as ticks <10 and ≥10 for use of logistic regression. Seropositivity in livestock species, as well human serologic results and reported symptoms were tested with the chi-square test (or where appropriate, Fisher's exact test was used).

To analyse the interaction between seropositivity in people and in livestock within camps, a generalised linear latent and mixed model (STATA command gllamm, Rebe-Hesketh, 2001) was used. This multilevel model allowed for inclusion of the denominator (number of people sampled per camp) in the analysis. The goodness-of-fit of the models (G) was assessed by the difference of the deviance (-2 log likelihood) of the model with the intercept only and of the final model, and a chi-square test was performed on the appropriate degrees of freedom to determine p-values.

3. Results

3.1. Samples

Blood samples could not be obtained from 80% of young children (<5 years), 20% of older children (5–14 years), 5% of adults aged 15–45 years and 15% of older adults ≥46 years due to difficulties with blood taking or refusal of participants or their mothers. Brucellosis-serology results were available for 860 humans and 1637 animals. A total of 368 human and 613 animal sera were tested for Q-fever. Complete questionnaire data was available from 710 people and for 1142 animals (Table 1).

3.2. Individual and camp seroprevalences of humans and livestock

A total of 28 brucellosis-seropositive human samples resulted in a true seroprevalance of 3.9% (Table 2). One of these sera showed titres for IgG- and IgM-antibodies. Four other sera had high IgM-antibody titres alone—indicating recent sero-conversion (Tizard, 1992). Positive results were more frequent for cattle (seroprevalence of 6.6%) than for camels and small ruminants (0.4 and 0%) (Table 2). Cattle kept by Fulani and Arabs were equally affected (RE logistic-regression model adjusted for sampling, cattle breed and age of cattle, $P = 0.5$). Two strains of *Brucella abortus* biovar 6 were isolated from two cow-milk samples. Brucellosis-seropositive animals were found in 14 out of

Table 1
Overview of the samples used in a survey of brucellosis and Q-fever in nomadic pastoralists and their livestock in Chad (1999–2000)

Age class (years) or species	Brucellosis		Q-fever	
	Serum samples	Questionnaire data[a]	Serum samples	Questionnaire data[a]
Fulani cattle breeders				
Humans				
0–4	18	0	16	0
5–14	64	24	36	32
≥15	399	374	158	155
Livestock				
Cattle	488	465	163	153
Goat	216	176	71	61
Sheep	206	160	78	63
Arab cattle breeders				
Humans				
0–4	0	0	0	0
5–14	9	2	3	2
≥15	72	71	6	6
Livestock				
Cattle	90	76	11	8
Goat	29	16	4	3
Sheep	34	24	5	3
Arab camel breeders				
Humans				
0–4	14	0	10	0
5–14	53	22	33	29
≥15	231	217	106	98
Livestock				
Camel	288	128	142	26
Cattle	30	18	21	19
Goat	129	34	59	3
Sheep	127	45	59	12

[a] Humans: age, sex, and risk behaviour. Livestock: age, number of births, and history of abortions.

20 camps with at least one seropositive person and in 3/5 camps with at least two seropositive persons. No significant correlation was found between human brucellosis serostatus and camp proportions of seropositive large animals (cattle and camels) and of small ruminants (generalised linear latent and mixed model, $G = 1.3$; d.f. $= 2$; $P = 0.5$).

Fifteen Q-fever seropositive blood samples were taken from 11 Arab camel breeders and 4 Arab cattle breeders. The human seroprevalence for Q-fever was 1%. However, two or more Q-fever seropositive camels were found in each camel herd, and individual seroprevalence in camels was 80%. (Table 2). No significant correlation was found between human Q-fever serostatus and camp proportions of seropositive animals (both $P > 0.1$).

Table 2
Individual and camp seroprevalences of brucellosis and Q-fever obtained in a survey in nomadic pastoralists and their livestock in Chad (1999–2000)[a]

Zoonosis	Age class (years) or species	Individual			Camp		
		N tested	% Positive		*N* tested	% Positive	
			Prevalence	95% CI		≥1 positive = cutoff	≥2 positive = cutoff
Brucellosis	Humans	860	4	2, 5	54	37	9
	0–4	32	5	0, 19		Not calculated	
	5–14	126	1	0, 9		Not calculated	
	≥15	702	4	2, 6		Not calculated	
	Camel	288	0.4	0, 2	17	24	0
	Cattle	608	7	4, 9	39	64	28
	Goat	374	0	–[b]	49	4	0
	Sheep	367	0	–[b]	46	4	0
Q-fever	Humans	368	1	0, 2	32	22	12
	0–4	26	4	0, 12		Not calculated	
	5–14	72	2	0, 5		Not calculated	
	≥15	270	2	0.1, 3		Not calculated	
	Camel	142	80	71, 87	14	100	100
	Cattle	195	4	1, 7	19	37	21
	Goat	134	13	7, 19	28	46	11
	Sheep	142	11	5, 16	28	43	14

[a] Apparent serorevalences were calculated with RE on the camp level and then transformed into true seroprevalences.
[b] Not applicable due to very low apparent seroprevalences and test characteristics.

3.3. Factors associated with human serostatus of *Brucella spp.* and *C. burnetii*

Factors associated with brucellosis and Q-fever seropositivity are presented in Table 3. There was a (weak) trend ($P = 0.11$) toward an association between male participants and brucellosis seropositivity (male seroprevalence 4.8 versus female seroprevalence 2.5). Being a camel breeder was a significant ($P = 0.03$) risk factor for Q-fever seropositivity in humans.

Virtually all participants (including children under 5 years), consumed raw milk (98%, 95% CI 96–99) and 62% (59–66%) said they had direct contact with placentas of livestock from time to time. No differences in the frequency of raw-milk consumption or the species producing the milk were observed between genders within camel or cattle breeders (data not shown). Most adult camel breeders (87%) consumed small-ruminant milk in addition to camel milk and 10% also consumed cattle milk bought at markets or from neighbouring camps. Sporadic direct contact with placentas was reported for 27% (21/77) of children aged from 5 to 14 years and was as high as 66% for adults. In total, 84% adults did obstetric work, and more men than women reported performing obstetric work on the livestock (RE logistic-regression adjusted for age category and ethnic group, $P < 0.0001$).

Table 3
Results of backward stepwise RE logistic-regression analyses of potential risk factors (explanatory variables) for *Brucella* spp. and *C. burnetii* seropositivity from a survey in nomadic pastoralists and their livestock in Chad (1999–2000)

Explanatory variable	Negative ($n = 683$)	Positive ($n = 27$)	OR	95% CI	$P(LRX^2)$
Brucellosis[a]					
Sex					
Females	307	8	1	–	
Males	376	19	2	0.8, 4.5	0.11
	Negative ($n = 310$)	Positive ($n = 12$)			
Q-fever[b]					
Breeding system					
Cattle breeder	192	3	1	–	
Camel breeder	118	9	9	1, 82	0.03
Sex					
Females	147	2	1	–	
Males	163	10	4	0.8, 21	0.07

[a] Brucellosis: $G = 2.5$, d.f. $= 1$, $P = 0.1$.
[b] Q-fever: $G = 8$, d.f. $= 2$, $P = 0.02$.

3.4. Clinical manifestation and zoonotic serostatus in humans and animals

Four out of five participants with brucellosis IgM-antibodies reported an illnesses of >1-year duration. Back pain around the kidneys was reported by three out of five. In comparison, only 70/847 other participants mentioned this symptom (Fisher's exact test $P = 0.005$). Results of physical examination were not different for IgM-seropositive and seronegative participants (all P from Fisher's exact test ≥ 0.2).

A total of 19% of brucellosis-seropositive cows had a history of abortion. Brucellosis seropositivity of cattle was significantly correlated to history of abortion (RE logistic-regression adjusted for age and breed, OR $= 2.8$; 95% CI 1.2–7; $P = 0.03$). Multiple abortions have been reported for 4 out of 10 seropositive cows with history of abortion. In our settings, carpal hygromas were only seen in two cattle herds and animals of these herds did not show higher brucellosis seroprevalences than animals in other camps.

Camels had a higher tick burden than cattle, with 19% of camels with ≥ 10 ticks (mostly in the perianal area) per animal versus 7% of cattle (RE logistic-regression adjusted for sampling, OR $= 3.5$; 95% CI 1.3–9; $P = 0.01$). The Fulani reported efforts to reduce the tick burden of their cattle using smoke, picking off ticks, or acaricide treatments.

4. Discussion and conclusions

The 3.5% seroprevalence for brucellosis in humans in our study indicates that this infection is endemic at a low level—comparable to other nomadic settings (Roth et al., in press). We did not identify any relationship between human seropositivity and seroprevalences of different livestock species in the same nomadic camps. Milk is a staple food in

nomadic pastoralist societies and is an important source of vitamin A for nomadic pastoralists (Zinsstag et al., 2002). On the other hand, uncooked milk—which was the way virtually all participants of our study consumed it—also is a source of infection with milk-borne zoonoses. Young children might have acquired brucellosis seropositivity through consumption of raw milk ($n = 2$). Furthermore, brucellosis can be acquired as an occupational hazard for nomads performing obstetric work. Men did most of the livestock obstetric work, which might explain why more men were seropositive for brucellosis in this study than in other sub-Sahelian populations (Gidel et al., 1974). The source of infection for camel breeders remains unclear. With regard to high-risk behaviour, we concluded that seroprevalences in humans were rather low (likely due to limited active foci in livestock). An unexpected high variability of inter-visit camp-member composition was observed. This dynamic of nomadic camps might partially explain the non-correlated relationship between seroprevalences in humans and in livestock. Only a weak association between clinical symptoms of people and serologic results also was observed by other authors for brucellosis (Lefèvre et al., 1970; Maichomo et al., 1998).

Nomadic pastoralists try to keep contacts between different livestock herds (including sedentary local herds) as low as possible. Nonetheless, animals are sometimes purchased or kept on behalf of "foreign" owners. The cattle herd with the highest mean brucellosis ELISA-values was composed of recently purchased animals (with unknown history of abortion). Our results on cattle seroprevalence (7%) are in accordance with other data obtained in the Sahelian zone of other African countries (Gidel et al., 1974; Akakpo and Bornarel, 1987). In contrast, an earlier sero-study of cattle in sedentary herds of southern Chad found seroprevalences between 20 and 30% (Domenech et al., 1982)—but seropositivity of cattle for brucellosis seems to be generally more prevalent in the more-humid regions south of the Sahel.

We found an association between any history of abortion and seropositivity of cows—an association often described in literature (McDermott and Arimi, 2002). Typical clinical signs of brucellosis in cattle are well known to Fulani nomads, but the Fulani did not associate the disease in cattle with that in humans (Krönke, 2001). Out of 49 Fulani questioned on the symptoms of the livestock illness *bakkale*, 12 mentioned swollen testicules, 20 infertility of cattle, 26 frequent miscarriages, and all 49 swollen knees (hygromas—but not all animals with hygromas are brucellosis sero-reactors; Perreau, 1956).

To our knowledge, the very-high Q-fever seroprevalence of camels (80%) has not been reported in the literature so far. Being a camel breeder was a significant risk factor for human Q-fever seropositivity. Afzal and Sakkir (1994; United Arab Emirates) and Elamin et al. (1992; Sudan) stated that camels are to be considered as an important source of Q-fever. However, Q-fever seroprevalence was as low as 1% in our study. Domenech et al. (1985)—who also observed a good knowledge of breeders on livestock abortions—stated that Q-fever (and chlamydiosis) only played a minor role in abortion compared to brucellosis. As a matter of fact, they found higher seroprevalences of Q-fever among animals with no history of abortion as compared to animals with miscarriages. This finding is in accordance with our study.

In general, the indirect ELISA is considered well suited for serological survey in non-vaccinated populations because laboratory variations are reduced in comparison to other tests (Samartino et al., 1999). It was not the primary aim of this study to assess the test

characteristics of the brucellosis ELISA for use on human sera; however, contradictory results of different serologic tests made further testing necessary to best evaluate the test characteristics. The use of a blocking or competitive ELISA (being species-antibody-independent) or (for the presence of brucellosis-anti-IgM-human sera), the use of a conjugate binding to human-IgG- and IgM-antibodies in the indirect ELISA would have been more appropriate (but was not available).

The sampling procedure allowed us to visit camps during the dry and wet seasons and, thus, season-dependent diseases such as malaria could be recorded. We decided to revisit the same camps at second and third sampling. The described procedure with selection of camps could not be newly established for the second sampling when nomadic groups were very dispersed in remote zones.

The dynamic of the composition of nomadic camps led to the selection of mainly new individuals (>85%) at second and third visit in the same camps. The variable sampling number was not associated significantly with the human serostatus (adjusted for sex, age class and group, $P > 0.1$). The period of seroconversion can basically not be determined by looking at IgG-antibody titres. Therefore, we decided to pool the three samplings after exclusion of second or third blood samples from same individuals. We had five IgM-positive samples, and four out of these were collected during the first sampling in the dry season. More-active brucellosis foci prior to the first sampling than during the following year (leading to increased IgM-titres) cannot be excluded. However, we do not think that this represents a seasonal variation because we should have seen the same trend during the next dry season. As to Q-fever, only sera and data of the first sampling have been evaluated.

In parallel to rearrangement of the composition of the camp-members, the camp's herd composition changed over time. Animals have not been marked, and thus the proportion of animals which have been sampled a second time is unknown. Almost exclusively females in lactation were sampled. At the first visit of a camp, the animal pointed out by the veterinarian sometimes was not captured; if so, a neighbouring animal for which the livestock owner wanted a treatment (most often against trypansomiasis) was presented—despite the previous explanations that no animal would be treated before the sampling procedure was completed. The names of each camel and cattle were requested to judge whether the information on age, parity and abortion should be recorded. In 80% of the cases, the owner (or a relative who knew the life history of his animals very well) was present. Recall bias and the unawareness of abortions during the first part of gestation must nevertheless be considered. We have no indication that livestock abortions are under-reported due to shame. In contrast, the painful nature of reporting human miscarriages can lead to an under-reporting, and any data relative to this topic have to be interpreted cautiously and were not evaluated any further.

The nomads' working rhythm allowed for examination and interviewing only in the early morning or in the evening before sunset; time per participant for physical examination and completion of the survey questionnaire (always by the same physician) was limited. Therefore, the number of questions on risk behaviour was limited. Complete individual questionnaire data was missing from 17% of participants for whom a blood sample was available. Furthermore, blood was only taken from 20% of children <5 years old. These missing data represent a source of bias (especially, the systemic missing of data on young children); however, the consequences for the final results of the study cannot be predicted easily. For example small children might have been less resistant to zoonotic infections

than adults—but, in contrast, only were exposed to one risk factor (consumption of raw milk) and had less direct contact to potentially infected animal material. Tick-infestation of livestock was not recorded during the first sampling and thus could not be compared to Q-fever serostatus.

Due to their marginalised status, nomads are mostly not considered for health interventions. Our study was directed towards acquisition of knowledge on morbidity of Chadian nomadic pastoralist communities and the development of health-care services adapted to the specific needs of nomadic populations. Three-forth of the participants had a complaint of respiratory tract disorders, alimentary disorders, or malaria (Daoud, 2001). The impact of the zoonotic diseases tested on the health status of the study populations seemed to be comparatively low. The present study helped to better appraise the relevance of zoonotic seroprevalences in humans and livestock and to start a fruitful intersectoral collaboration between the Chadian public-health and veterinary sectors following the concept of "one medicine" (Schwabe, 1984). Bovine and human tuberculosis (with a special focus on tuberculosis caused by *Mycobacterium bovis* among human patients) currently is being evaluated in the first mycobacteria laboratory in Chad. Joint human and animal vaccination campaigns among nomadic communities have been established, for which public-health agents join the veterinary teams during compulsory cattle vaccination to vaccinate nomadic children and women in remote pastoral areas. Health-information campaigns are grafted upon these vaccination activities, whereby information on the hazards and prevention of zoonotic diseases can be communicated to a larger population.

In conclusion, human seroprevalences of brucellosis and Q-fever were relatively low in nomadic pastoralist communities of the Sahelian zone in Chad, although seroprevalences of brucellosis in cattle was 7% or of Q-fever in camels 80% and most nomads reported high-risk behaviours such as consumption of raw milk or contact with aborted livestock material.

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