Brucellae through the food chain: the role of sheep, goats and springbok (*Antidorcus marsupialis*) as sources of human infections in Namibia

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ABSTRACT

A confirmed case of human brucellosis motivated an investigation into the potential source of infection in Namibia. Since domestic animals are principal sources of *Brucella* infection in humans, 1692 serum samples were screened from sheep, goats and cattle from 4 presumably at-risk farms and 900 springbok (*Antidorcas marsupialis*) serum samples from 29 mixed farming units for *Brucella* antibodies by the Rose-Bengal test (RBT) and positive cases confirmed by complement fixation test (CFT). To assess the prevalence of human brucellosis, 137 abattoir employees were tested for *Brucella* antibodies using the standard tube agglutination test (STAT) and by enzyme linked immunosorbent assay (ELISA). Cattle and sheep from all 4 farms were negative by RBT and CFT but 2 of the 4 farms (Ba and C) had 26/42 and 12/285 seropositive goats, respectively. *Post mortem* examination of seropositive goats revealed no gross pathological lesions typical of brucellosis except enlarged mesenteric and iliac lymph nodes seen in a single buck. Culture for brucellae from organs of seropositive animals was negative. None of the wildlife sera tested positive by either RBT or CFT. Interviews revealed that besides the case that prompted the investigation, a family and another person from other farms with confirmed brucellosis shared a common history of consumption of unpasteurised goat milk, home-made goat cheese and coffee with raw milk and prior contact with goats, suggesting goats as the likely source of infection. All 137 abattoir employees tested negative by STAT, but 3 were positive by ELISA. The 3 abattoir workers were clinically normal and lacked historical connections with clinical cases. Although goats are often associated with *B. melitensis,* these studies could not explicitly implicate this species owing to cross-reactivity with *B. abortus,* which can also infect goats. Nevertheless, these data reinforce the need for a better National Control Programme for brucellosis in Namibia.

Keywords: abattoir, brucellosis, goats, Namibia, serology.

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INTRODUCTION

The emergence or re-emergence of zoonotic diseases is complex and multifactorial, often driven by evolving ecology, microbial adaptation, human demographics and behaviour, international travel and trade, agricultural practices, technology and industry[49]. The World Organization for Animal Health (OIE) is mandated under the Sanitary and Phytosanitary Agreement (SPS) of the World Trade Organization (WTO) to develop minimum scientific or evidence-based international standards, guidelines and recommendations to facilitate safe trade in animals and their products[51]. In the case of zoonotic diseases such as brucellosis, it is believed that protection of human health can be achieved through control of the disease in the animal population. The OIE/FAO (Food and Agriculture Organization)/WHO (World Health Organization) Global Early Warning System (GLEWS) provides for rapid notification of major animal diseases, including zoonoses, of which *B. melitensis* infection is a priority disease[51].

Brucellae are small, non-motile, Gram-negative coccobacilli that are responsible for one of the most widespread zoonotic infections of medical significance worldwide[28,44]. Humans are accidentally infected through direct or indirect contact with infected material and are invariably dead-end hosts of *Brucella* infections, while some wildlife species can act as potential reservoir hosts[15,17,19]. The pathogenic species are *B. melitensis,* which predominantly infects goats and sheep; *B. abortus,* which principally affects cattle; *B. suis* that infects swine and *B. canis* that infects dogs[5,11,21,52,53]. Although any of 4 species of *Brucella* can cause systemic disease in humans, *B. melitensis* has the lowest infective dose, requiring as few as 10 organisms to cause infection[2,9,25]. Human brucellosis, commonly referred to as undulant fever or Malta fever, often coincides with livestock infection[4,35]. The disease in humans presents as a multi-systemic, acute to chronic disease characterised by non-specific signs such as fever, headache, joint pains, musculoskeletal pains, sweating, malaise, myalgia, abdominal pain, lymphadenopathy, skin rash, pneumonitis, back-ache and body wasting[9,21,25,36]. The non-specific nature often presents a tremendous challenge in clinical diagnosis of brucellosis since these signs can also occur in common diseases or conditions like malaria, typhoid, rheumatic fever and pyrexia of unknown origin[21]. Unlike in Tanzania[25,45], the disease's status in Namibia is not precisely known as most health centres do not routinely test for brucellosis. Sporadic cases and even small clusters are often difficult to identify owing to an extremely variable incubation period (weeks to months) and a lack of pathognomonic clinical features or manifestations[27,44].

Of the zoonotic species, *B. melitensis* contributes up to 70 % of human brucellosis cases worldwide[14,32] with sheep, goats and camels being the main sources of infection[9,24]. The difficulty in clinically diagnosing brucellosis, coupled with the weakness of human health services in developing countries, often contribute to human brucellosis being inadequately treated[34]. Bacteria gain entry into the body by ingestion, inhalation, penetration through skin abrasions as well as through conjunctival mucosa[41]. Once in the body, *Brucella* spp. survive and multiply within cells of the reticuloendothelial system. Notably, human neutrophils exhibit variable antiphagocytic activity

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towards some *Brucella* strains but are virtually ineffective against *B. melitensis*[52,53]. However, once excreted, brucellae rarely survive for long periods in tropical environmental conditions. Risk factors for infection in humans include handling of infected dead or live animals, ingestion of contaminated animal products especially unpasteurised dairy products, poor handling of Rev 1 vaccine and cultures of *Brucella* spp[2]. The Rev 1 vaccine is potentially harmful to humans. Vaccinated animals should not be slaughtered for human consumption within 3 months of vaccination. Although it is believed that with ordinary handling this vaccine is harmless to man , care should nevertheless be taken not to accidentally inject it into humans and contamination of human eyes with this vaccine should be avoided. By virtue of increased contact with animals and their products, abattoir workers are at a greater risk of brucellosis infection compared with other professional groups[2,45]. While notification of diseases in wildlife is not a pre-condition for imposing bans on members of the WTO who implement the OIE terrestrial code[35,53], the ever-changing game farming systems create a need to establish and distinguish between a spill-over infection from domestic animals and maintenance of infection in wildlife species.

A single human case of undulant fever or Malta fever was suspected in a community surrounding the interface of wildlife farming and domestic animals in Namibia. At clinical examination, brucellosis was considered a differential diagnosis and a blood sample was collected for serological testing at a national laboratory in Namibia and at the National Institute for Communicable Diseases, Johannesburg, South Africa. The serum sample tested positive for *Brucella* IgG and IgM antibodies by enzyme linked immuno-sorbent assay (ELISA), providing a definitive diagnosis of brucellosis. This prompted the undertaking of a large serological survey to provide evidence of brucellosis in domestic animals and springbok (*Antidorcus marsupialis*), a small ungulate frequently found in close proximity to farmed livestock and handled by abattoir workers. The findings suggest direct or indirect contact with goats or their unpasteurised products as the most likely source of infection to humans, while farmed wildlife presented the least likely threat.

MATERIALS AND METHODS

Background data, study area and population

A human case of brucellosis in February 2009 at a commercial farm in Hardap region triggered an investigation into the potential source of infection in this area and 3 other surrounding regions in the foot-and-mouth disease-free zone, namely Karas, Omaheke and Khomas (Fig. 1). The main farming activities in these regions include extensive sheep, goat and game rearing. These regions are home to 88.3 % of the 2.7 million sheep in Namibia. A single abattoir caters for animals from these 4 regions and has an annual turnover of 219 929 sheep and 6251 springbok (*Antidorcas marsupialis*). Relevant background data in the study regions were collected via a questionnaire that included the following main categories: i) vaccination with Rev 1, ii) animal trace-back and trace-forward movements to and from the suspected farms within a year, iii) farms and other places visited by family members within a year, and iv) sources and type of animal products consumed. Farms presumed to be at risk were identified by the track back system and designated A, B, C and D. These farms held varying numbers of small ruminants and farmed wildlife. Information retrieved from the computerised Namibian Livestock and Traceability System (NAMLITS) indicated that farm A had 2 milking Jersey cows, 130 Boer goats and 1300 sheep. The farm serum samples had previously been tested using RBT and declared brucellosis-free through annual serological testing. Farm B was inherited and had been subdivided into portions Ba and Bb between family members. Portion Ba held 984 sheep comprising of Dorper, Damara and Persian breeds, 39 Boer goats and 3 Saanen dairy goats, and portion Bb had 160 Boer goats and 11 Saanen dairy goats. Farm C held 285 Boer goats and an undisclosed number of sheep. Farm D held 106 dairy goats; the largest Saanen dairy goat operation in the region, which supplied most of the milk to all 4 regions. Goat movement from farm Ba's grazing camps within 1 year from the date of initial investigation to farm C was tracked through the Namibia Livestock Traceability system (NAMLITS). However, discrepancies in the actual numbers of animals were noted at some of the farms at sampling. A human case of brucellosis that triggered the investigation resided in farm A. Commercial harvesting of springbok was done in 3 of the 4 regions.

To examine the trend of this infection over previous years, data on the reported serological incidences of brucellosis using Rose-Bengal test (RBT) and complement fixation test (CFT) in animals between 2004 and 2009 in Namibia were retrieved and analysed.

Sampling techniques

The sample size for farmed wildlife was determined by the formula: $n = \log\{\alpha\}/\log(1 - p_{max} \times \text{sensitivity})$, where n = the required sample size, p = the prevalence (based on livestock data), α = the probability (confidence level) of missing a diseased animal at a prevalence of up-to p_{max} in a large population. The population size refers to the total number of springbok in the 3 regions exercising commercial harvesting. The sampling protocol was designed to detect at least 1 positive animal in the flock at a 95 % confidence limit, that is, if the disease was present at a prevalence (p) of 5 % in harvested springbok when the test sensitivity is 95 % and animals are randomly selected. In this particular study on farmed wildlife, the 3 regions were defined as an epidemiological unit. A total of 900 adult springbok from farmed flocks in 3 regions were sampled by systematic random sampling where a starting and interval number was picked at random from 1 to 8 during commercial harvesting operations with a minimum farm blood sample size of 11. Only adult male and female animals were selected for harvesting.

For sheep and goats, the sample size was determined by the formula: $n = \{1 - (1 - a)^{1/D}\}\{N - (D - 1)\}/2$ as described in Cannon and Roe[3] where n is the required sample size, a is the error probability (confidence level) of observing at least 1 diseased animal when the disease affects at least D/N in population, D is the number of diseased animals in a population and N is the population size. The flock size was in reference to the number of animals in the group being sampled. The sampling protocol was designed to detect at least 1 positive animal in the flock at a 95 % confidence limit, that is, if the disease is present at a prevalence of 5 % in animals of breeding age (6 months and older). If an animal was picked and not subjected to blood collection, it was placed in a different holding pen.

Preparation of serum from domestic animals and farmed wildlife

The sample sizes of domestic ruminants differed from farm to farm. All animals were sampled by venipuncture of the jugular vein. On farm A, 77 sheep, 25 goats and 2 milking cows were bled. On farm subdivision Ba, 39 goats and 984 sheep were bled while 34 goats were bled on subdivision Bb. After initial positive results of samples from farm Ba, all 285 Boer goats on farm C, all sheep and goats on farm Ba and 106 goats from farm D were bled and serologically tested for brucellosis. Blood was allowed to clot at ambient temperature and placed in a

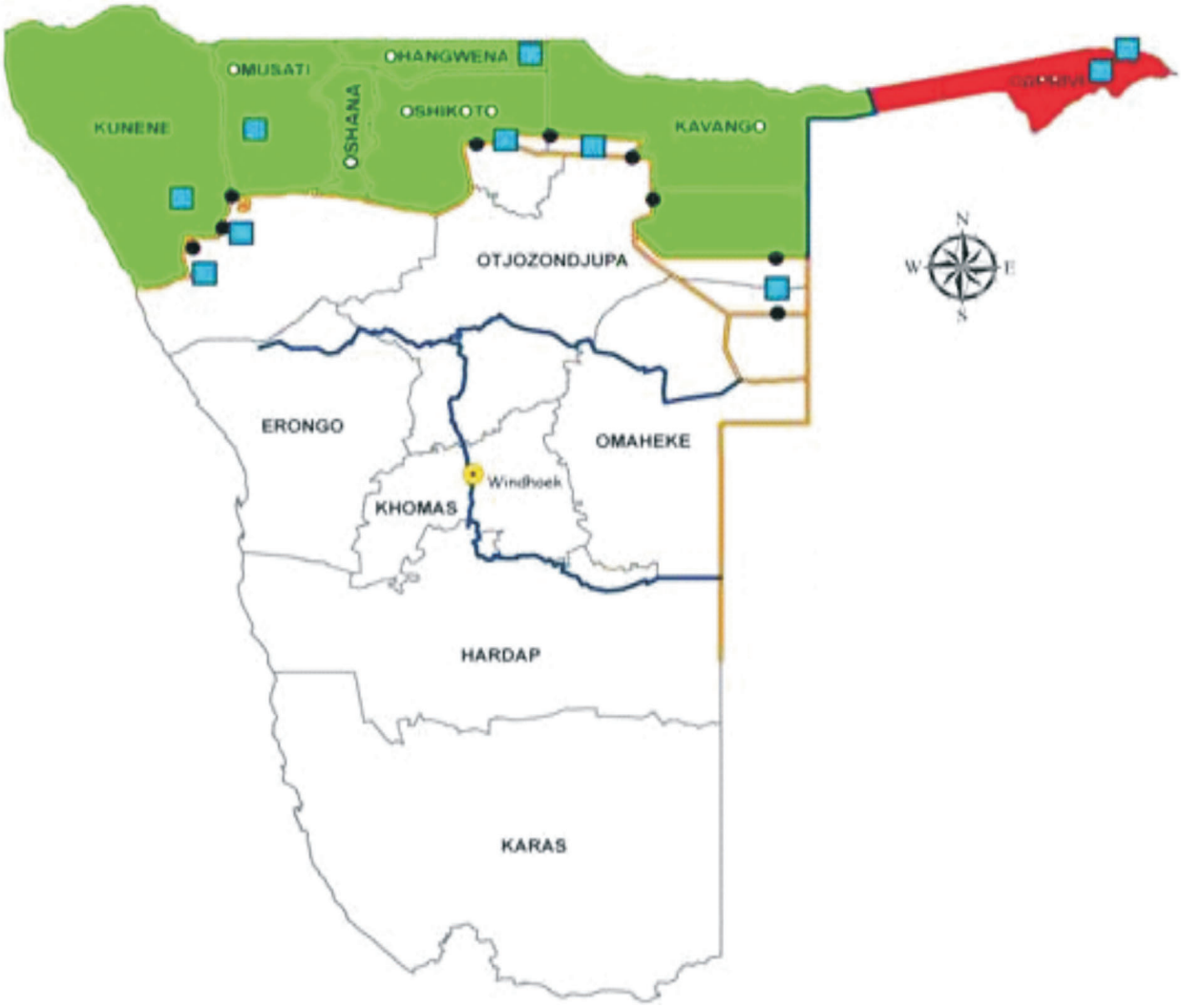

Fig 1: **The areas under study** (Khomas, Omaheke, Hardap and Karas) **in relation to foot-and-mouth disease-infected and protected areas** (shaded grey)**.**

cooler box for transit to the laboratory. Serum was separated from the clot by centrifugation (13 000 *g*) for 10 min, collected into fresh tubes and stored frozen at –20 °C until required for analysis.

Blood samples were collected randomly from springbok at harvesting periods; April to August 2009 and July to August 2010. Typically, blood was freely collected into test tubes upon severing of both carotid and jugular vessels, allowed to clot at ambient temperature and then placed in a cooler box before processing. Sera were prepared, collected and stored in a similar manner as described above for domestic animals.

Serological testing of domestic animals and farmed wildlife

All sera were submitted to the Central Veterinary Laboratory (Windhoek) for serological testing essentially following standard protocols[1]. In the serial testing, it was assumed that both the RBT and CFT are 90–91.8% sensitive and 99–99.9% specific at 95 % confidence in detecting *Brucella* antibodies in sera from culture-positive animals. Sera from domestic animals and farmed wildlife were initially screened for brucellosis by the RBT and positive serum samples were verified by CFT. Reference antigens for *Brucella* species were obtained from Onderstepoort Biological Products (Onderstepoort, South Africa) while the reference anti-sheep red blood cell antibody (Amboceptor) and complement were procured from Siemens Healthcare Diagnostics Products GmbH (Erlangen, Germany). Briefly, a 2-fold serial dilution of complement-inactivated animal sera and reference positive (Amboceptor) and negative controls were prepared in Veronal buffer as per the manufacturers' instructions. An equal amount of an appropriately diluted standard antigen was added to all wells followed by complement. Complement activity was detected after addition of washed 3 % sheep red blood cells.

Serological testing of abattoir employees

To assess the prevalence of brucellosis in humans sera were tested from abattoir employees (Farmers Meat Market abattoir, Mariental, Namibia). The sera were collected as part of the abattoir's occupational health surveillance and regulatory monitoring of workers' health in the meat industry. Approval to use such sera was obtained from the abattoir's management in consultation with their medical doctor and the workers' representatives. A total of 137 human serum samples representing individuals involved in buying, slaughtering and deboning small ruminant and game animal carcasses were obtained. The relative proportions of each age group and gender of the employees sampled are given in Table 1. Serum samples were processed at an accredited laboratory (Pathcare, Windhoek, Namibia) and stored at –20 °C prior to use. Serum samples were screened by the standard tube agglutination test (STAT) using *B. abortus* and *B. melitensis* antigens (Linear Chemicals SL, Spain) as per the manufacturers' instructions where a titre of 1:80 or greater was considered positive. Positive samples were confirmed by enzyme-linked immuno-sorbent assay (ELISA) using the Panbio *Brucella* IgG

Table 1: **Distribution of abattoir workers tested for brucellosis on the basis of age and gender.**

Age (years)	Gender				Total		Positive for brucellosis
	Male		Female		Number	%	
	Number	%	Number	%			
<20							
20–30	31	22.63	6	4.38	37	27.01	0
30–40	63	45.99	11	8.03	74	54.01	1
40–50	19	13.87	2	1.46	21	15.33	2
50–60	4	2.92	1	0.73	5	3.65	0
>60	0	0	0	0	0	0	0

ELISA kit (Plasmatec Laboratory Products, Dorset, UK).

All seropositive animals were slaughtered separately at a non-export local municipality abattoir at the end of the slaughter week and subjected to *post mortem* examination according to local health and safety guidelines for handling suspected zoonotic cases. Tissue samples were collected from the liver, kidney, spleen, lymph nodes (iliac, oropharyngeal, inguinal, mesenteric and supramammary), testicles and udder for the detection and isolation of brucellae. Where pregnant animals were involved, the foetal lung and abomasal contents, uterus, and placenta membranes were sampled and processed following standard techniques[1]. No abortive material or vaginal discharges were obtained. Briefly, impression smears were made from a cut surface of the organs by 1st blotting on clean absorbent paper and then gently pressing onto a clean glass slide. Smears were air-dried and heat-fixed before staining by a modified acid fast technique. Fixed smears were flooded with dilute carbol fuchsin for 10 min, rinsed in tap water, differentiated with 0.5 % acetic acid for 30 s and counterstained with 1 % methylene blue for 20 s. Slides were air-dried and then viewed with a light microscope under oil immersion.

Tissue samples were cultured on agar on Farrell's selective medium for brucellae[13]. Briefly, samples collected at *post mortem* were homogenised in sterile phosphate buffered saline (PBS) using a stomacher before being inoculated onto the solid media. Aliquots of 100 mℓ of each tissue homogenate were streaked onto *Brucella* selective agar and the plates incubated aerobically in enriched CO_2 with daily examination for brucellae colonies up to 7 days.

RESULTS

Data from questionnaire

Interviews revealed that apart from the brucellosis positive individual who triggered the investigation, 3 additional family members from the Hardap region (where farm A is located) and an unrelated person from another region had previously been treated for suspected Malta fever. Interestingly, all humans diagnosed with brucellosis shared a common history of consuming raw goat milk, home-made goat cheese and coffee with raw milk and prior contact with dairy goats. None of the farms used Rev 1 vaccine in sheep or goats, but heifers were vaccinated with *B. abortus* strain 19. Reported clinical signs suggestive of brucellosis in small ruminants of farms A and C included abortions, orchitis, and arthritis whereas those seen in dairy goats of farm B were abortions, retained placenta and arthritis.

Unpasteurised cow milk from farm A was mainly consumed on the farm with small quantities occasionally sent to farm B. Raw goat milk was consumed at subdivisions of farm B at least once per week. In general, the substantiated links between farms A and B were i) cow milk ii) individuals from farm A visiting and drinking coffee with unpasteurised goat milk on farm B and iii) a ram introduced onto farm A from farm B. It was not possible to substantiate clear links between these farms (A and B) and farms C or D, apart from movement of people between them. Nevertheless, these findings provided some clues on the possible route of transmission of brucellosis to humans.

Serological testing of domestic ruminants and wildlife

Samples of sheep (n = 77) and goats (n =25) from farm A and farm subdivision Bb (n = 34) were serologically negative for brucellosis using the RBT. Initial serological analysis of 39 sera from farm Ba revealed 20 positive cases by CFT where a titre of 1:8 or greater was considered positive, prompting the testing of the entire flock on that farm and the nearby farm C, to explore the extent of the problem. Twenty-six out of 42 goats from farm Ba and 12 of the 285 from farm C were positive for brucellosis using the CFT. The positive cases on farm Ba included all milking goats while all sheep from both farms were negative. Of the 12 positive goats on farm C, 6 were pregnant females and a single 6–9-month-old male goat, whereas none of those on farm Ba were in lamb. The 2 Jersey cows on farm A were also negative by both serological tests. To contain the disease, farms B and C were immediately quarantined and the positive goats were isolated before being sent for slaughter at a local non-exporting municipality abattoir.

To investigate the disease status of farms Ba and C after quarantine and slaughter of positive goats,, the remaining animals were re-tested after 3 months and none tested positive for brucellosis, suggesting that the infection had been controlled. To explain the possible cause of orchitis reported on farm C, the sera of uncastrated males were tested for *Brucella ovis* antibodies by RBT and CFT and 3 positive cases were detected. *B. ovis* is of exclusive concern in rams where it causes epididymitis[20,9].

The annual reporting of the incidences of brucellosis in Namibia has previously focused on livestock but not farmed wildlife. With game animals increasingly entering the human food chain, the springbok and gemsbok (*Oryx gazella*) were 1st included in the reporting system in 2009, but the incidence was zero (Table 2). To determine whether farmed wildlife play a role in the transmission of brucellosis, a total of 900 springbok samples harvested from 29 different farming units were tested, but none was positive by either RBT or CFT, suggesting that this species was not responsible for transmission of brucellosis to humans. These findings were consistent with the absence of clinical or pathological signs suggestive of brucellosis. It was therefore considered that no further action was necessary.

Serological testing of humans

Of the 137 serum samples screened for *Brucella* antibodies, all were negative by STAT, 3 were positive for IgG antibodies but negative for IgM antibodies by ELISA. The distribution of tested abattoir employees on the basis of age and gender is shown in Table 1. The positive samples were from males aged 31(1) and 40(2) years. These individuals were clinically healthy and received no treatment. To determine whether new cases developed, the same 137 individuals were bled as part of annual health screening and were re-tested for *Brucella* antibodies by ELISA and none was positive, indicating that the infection had cleared.

Table 2: **Number of brucellosis-positive samples from food-producing animals as determined by the Rose-Bengal test and complement fixation test from 2004 to 2009 in Namibia. Data were collected from the Central Veterinary Laboratory, Windhoek.**

Year	Species samples tested	Total number of samples	Number of positive	% seropositive
2004	Dairy cows	4001	19	0.47
	Other bovine	311	11	3.54
	Sheep and goats	18485	19	0.10
2005	Dairy cows	2541	2	0.08
	Other bovine	246	0	0.00
	Sheep and goats	10191	15	0.15
2006	Dairy cows	2994	9	0.30
	Other bovine	401	15	3.74
	Sheep and goats	3452	21	0.61
2007	Dairy cows	1578	0	0.00
	Other bovine	587	5	0.85
	Sheep and goats	1486	31	2.09
2008	Dairy cows	Data unavailable	N/A	N/A
	Other bovine	1910	15	0.79
	Sheep and goats	13745	37	0.27
2009	Dairy cows	Data unavailable	N/A	N/A
	Other bovine	1030	20	1.94
	Sheep	7376	4	0.05
	Goats	516	32	6.18
	Springbok and gemsbok	122	0	0.00

Post mortem findings and bacteriology

The goats were transported under a special movement permit to the abattoir and slaughtered in accordance with local Health and Safety regulations relating to the handling of zoonotic agents. Not surprisingly, the majority of seropositive goats were over 3 years old (Fig. 2). *Post mortem* analysis and collection of tissue samples for bacteriology was undertaken by the official state veterinarian following local instructions and precautions for handling zoonotic agents. *Post mortem* examination of all seropositive goats revealed no typical brucellosis lesions except in a single ram that had swollen mesenteric and iliac lymph nodes. Direct microscopy on collected organs failed to reveal any bacterial cells suggestive of the presence of brucellae. Subsequent culture of homogenised tissues also failed to yield any *Brucella* colonies after 7 days of incubation, suggesting the absence of an active infection in the seropositive animals.

Retrospective analysis of incidences of brucellosis in domestic animals and humans

To gain insights into the trend of brucellosis in farm animals and humans, annual records for the period 2004 to 2009 were retrieved and analysed. Seropositive bovine cases were found to be relatively high for the period under consideration, suggesting that brucellosis is endemic in domestic animals. The incidence of brucellosis in sheep and goats was relatively low from 2004 to 2008. An increase in the number of goat cases was observed in 2009 (Table 2), indicating goats as a likely leading source of brucellosis during this period. However, there were no corresponding data in humans for the same reporting period to indicate a zoonotic transmission. Human cases were only reported for the period 1997 to 2003 (Fig. 3), suggesting either a break in the reporting system or absence of cases until those triggering the present investigation.

DISCUSSION

The annual reporting of the incidences of brucellosis in domestic animals in Namibia (Table 2) relies on the RBT and CFT on sera from suspected cases. However, due to the cross-reactivity and potential interspecies infection by *B. abortus* and *B. melitensis,* it is difficult to ascertain the actual prevalence of each species. Differentiation of the species requires successful isolation and subsequent molecular characterisation studies[42]. Indeed, a multiplex PCR assay for the identification and differentiation of all *Brucella* species including conventional vaccine strains now exists[16] but requires *Brucella* colonies. Such studies were impossible in our study since we were unable to isolate *Brucella* from tissues of seropositive cases. Failure to obtain any *Brucella* colony from seropositive could have been due to the inactive infection in the animals as evidenced by lack of pathological lesions or the suppressive effect of the selective medium used. Farrell's medium has been reported to be inhibitory for *B. ovis* and some *B. abortus* and *B. melitensis* strains[29]. Despite this, the selective advantage conferred by cycloheximine and a range of antibiotics makes this medium a priority for primary isolation of *B. abortus* from contaminated samples like *post mortem* tissues. Importantly, we have previously been successful in isolating of brucellae from organs of seropositive goats from other regions of Namibia using this medium, thus confirming its quality. Although alternative selective media for the isolation of brucellae have been described[7,23,26,43], these have not been widely used. Non-selective media like blood agar were not employed since brucellae are often overgrown by contaminating fungi and other fast-growing bacteria when plates are incubated for 7 days.

Brucellae are usually abundant in abortive material and vaginal discharges so these are highly recommended for the demonstration of brucellae by direct microscopy or culture methods. Although demonstrating the presence of *Brucella* organisms in tissues and fluids is central to confirming the diagnosis of brucellosis, serological testing remains the method of choice for estimating the prevalence of the infection in either humans or animals. It has recently been suggested that i-ELISA can be used for screening cattle with improved specificity, but the RBT and CFT remain confirmatory tests[31]. The RBT and CFT are conventional tests widely used in serological diagnosis of brucellosis in domestic animals where their respective sensitivities have been reported to correlate with culture-positive animals[8]. Application of the RBT and CFT serially with a simultaneous consideration of the test results increases the likelihood of detecting infected animals[39,48]. In humans, ELISA is the gold standard test

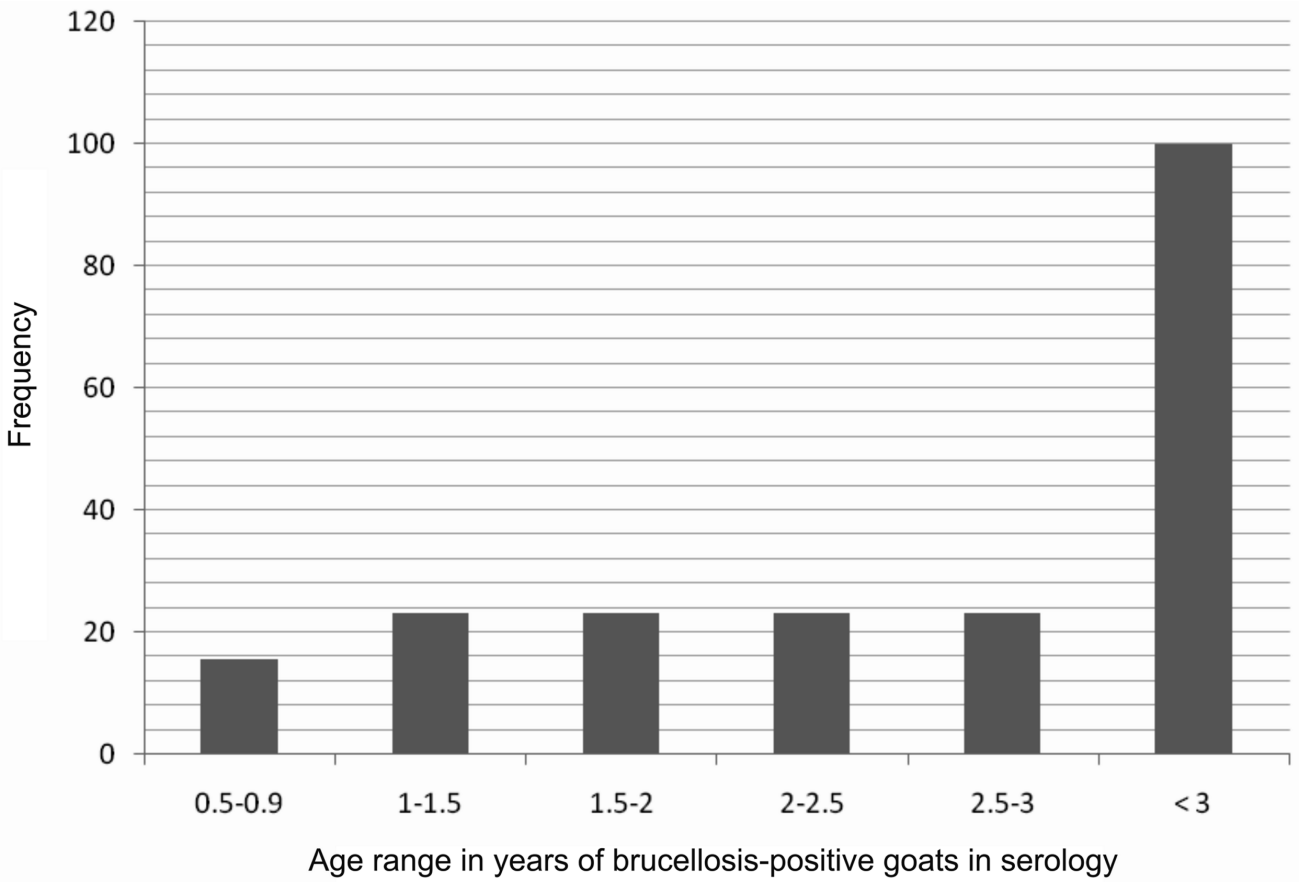

Fig. 2: **Cumulative frequency from different age categories of brucellosis-positive goats from 2 farms.**

for diagnosis of brucellosis, where its specificity is increased when IgM antibodies are not detected[50], although a conflicting report suggests its potential to detect false positives[10]. However, STAT has previously been reported to differentiate between active and inactive infections in humans based on the titres[54]. An immunocapture-agglutination test (Brucellacapt) also exists[37], but this is not widely used. The inability to detect any positive case with STAT suggests these

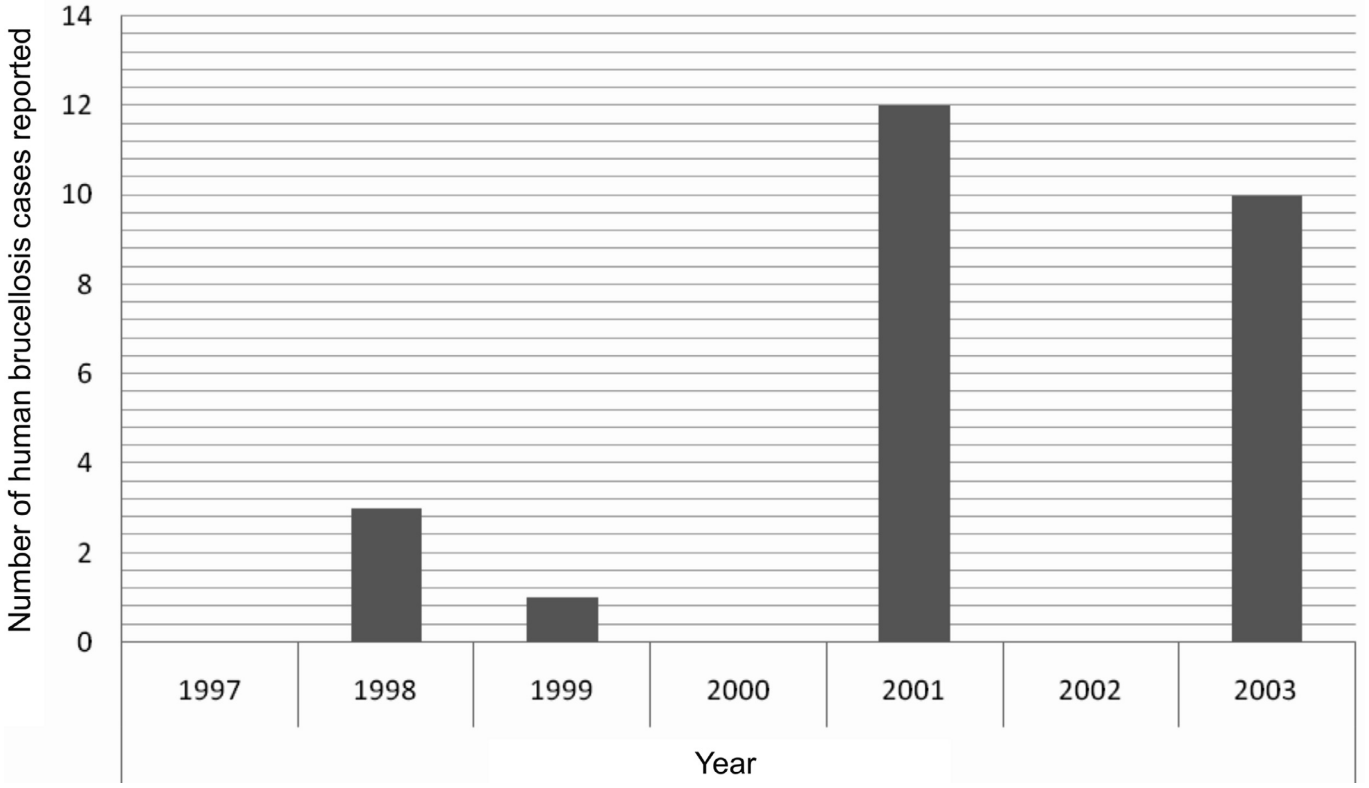

Fig. 3: **Number of official reports of human brucellosis in Namibia from 1997 to 2003** (OIE, Handistatus 11, http://www.oie.int/hs2/)**.**

were inactive infections, which was later confirmed by re-testing. In general, caution should be exercised in interpreting these serological tests, particularly in patients with chronic brucellosis, re-infections and relapses, and in endemic areas where a high proportion of the population carries antibodies against brucellosis.

Although brucellosis is rarely reported in wildlife, cases believed to be a spillover from small ruminants have been reported in chamois (*Rupicapra rupicapra*) and ibex (*Capra ibex*) in Europe[15,17,22]. Farmed springbok were therefore tested for brucellosis since these were reared in close proximity to small ruminants, which could result in a spillover of the infection. However, seroprevalences of 23 % and 48 % have been reported in African buffalo (*Syncerus caffer*) populations in the Kruger National Park and Zimbabwe respectively, where contact with domestic sheep, cattle, pigs and goats was absent[6,12,22]. Furthermore, several species of wildlife including African buffalo, zebra (*Equus zebra*), impala (*Aepyceros melampus*), waterbuck (*Kobus ellipsiprymnus*), and hippopotamus (*Hippopotamus amphibius*) have tested positive for *B. abortus* and *B. suis* worldwide, with *B. abortus* biovar 1 being isolated in the cotyledons of pregnant buffaloes[19,22]. In some areas where *B. abortus* had been eradicated in cattle populations, sheep and feral pigs have been implicated as sources of *B. melitensis* or *B. suis* infection respectively in cattle [6,12,47].

It was unclear why none of the seropositive goats, including pregnant females, failed to show typical lesions of brucellosis on *post mortem* examination.It was, however, not possible to exclude the possible presence of other cross-reacting pathogens. False-positive cross-reactions due to *Yersinia enterocolitica* serotype O:9 infection have been reported in cattle[18,39] . Another reason for seropositivity could be vaccine-induced antibodies, although the background investigations revealed that vaccination of goats with Rev 1 was not routinely undertaken on these farms. However, the enlarged lymph nodes found in the male goat was attributed to *B. ovis,* suggesting the presence of multiple infections within the flock. Based on the historical data gathered through a questionnaire and interviews, the animals testing positive for brucellosis, and a few abattoir employees also testing positive, it is strongly suspected that goats are the most likely source of infection. Although this could not be ascertained, it is likely that *B. melitensis* was involved, as it is usually associated with goats, since vaccination against *B. abortus* was routinely done in cattle. The consumption of unpasteurised goat milk or its products like home-made cheese is a leading antecedent to human brucellosis[33,38,40].

Where there is lack of traceability of animal movements to and from the affected farms, it is frequently impossible to trace the origin of the infection[30]. In this study, lack of a coordinated approach during the brucellosis case investigation by the Ministry of Health, State Veterinary region and Central Veterinary Office precluded effective planning and formulation of common strategies for food safety and control of zoonotic diseases. Consequently, there was a direct conflict in the implementation of simple preventative measures against brucellosis in abattoir workers that are usually exposed to a greater risk of infection despite the existence of health and safety related handling of suspected zoonotic agents. Although contact with infected herds and/or with contaminated environmental sources has been shown to play a major role in the spread of brucellosis, and despite historical sporadic outbreaks of brucellosis in humans, sheep and goats attributed to *B. melitensis,* the contribution of these and other factors to the epidemiology of brucellosis in game species in Namibia remains unclear. Consequently, more work is required to dissect the cross-species and within species transmission dynamics of brucellosis at the interface of wildlife and domestic animals.

In conclusion, the data highlight the risk of *Brucella* transmission through goats and unpasteurised products to humans. It is very likely that other human brucellosis cases were missed since the manifestations are non-specific. Therefore, a better control programme that includes public awareness, vaccination of goats with the Rev 1 vaccine (in which the native hapten-based gel precipitation test would have to be used to distinguish the serological response of infected animals from those induced in Rev 1 vaccinated animals) and pasteurisation of milk should be expected to reduce the incidences of brucellosis in humans.

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