# Survey of the seroprevalence of brucellosis, Q fever and Crimean-Congo haemorrhagic fever in humans and livestock in Herat province, Afghanistan

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## Introduction

Brucellosis caused by *Brucella melitensis* and *B. abortus* is endemic in Afghanistan but information about its occurrence is sparse and not easily verifiable.

A total of 39 confirmed cases of Crimean-Congo Haemorrhagic Fever (CCHF) were reported in Afghanistan between 2007 and 2012 and of these 24 occurred in Herat Province. The case fatality rate was 38.4% and 25 of the 39 cases had previous contact with animals. A study conducted in 2009 in the same district as a 2008 outbreak in 2008 found 11.2% of householders, 79.1% of cattle and 75% of sheep were seropositive (Mustafa et al., 2011).

Q fever is also endemic in Afghanistan and its public health importance was highlighted after reports of severe outbreaks in Bamyan province in 2011.

This study aimed to provide information with regard to the prevalence and concurrent status of brucellosis, CCHF and Q fever in humans and livestock in 10 randomly selected villages in Herat Province, and to investigate risk factors for their occurrence.

## Objectives

1. Determine the seroprevalence of brucellosis, CCHF and Q fever in village householders and their adult female sheep, goats and cattle in 20 randomly selected households in each of 10 villages in Herat province.
2. Identify putative risk factors associated with seropositivity for brucellosis and Q fever in householders and their livestock.
3. Determine householders' understanding of brucellosis and occurrence of husbandry practices which could influence levels of infection in animals and humans.
4. Recommend disease control measures based on the epidemiological findings of the study.

## Methods

The survey was conducted in 11 randomly selected villages (Figure 1) in 6 secure districts in Herat from 26 December 2012 – 17 January 2013. Six of the villages were Kuchi (transhumant or semi-nomadic pastoralists) and 5 were Sedentary villages with husbandry systems that did not involve seasonal migration.

A series of preparatory workshops were conducted to train study personnel in procedures for collection, processing, transport and storage of human and animal blood samples and administration of questionnaires. An inception workshop was held to inform local stakeholders and a motivation team visited the selected villages before commencing sampling activities in order to gain the support of village elders and obtain lists of householders. A comprehensive questionnaire designed to collect data on Knowledge, Attitude and Practice (KAP) at the household level was conducted by an interview with a senior person in each household.

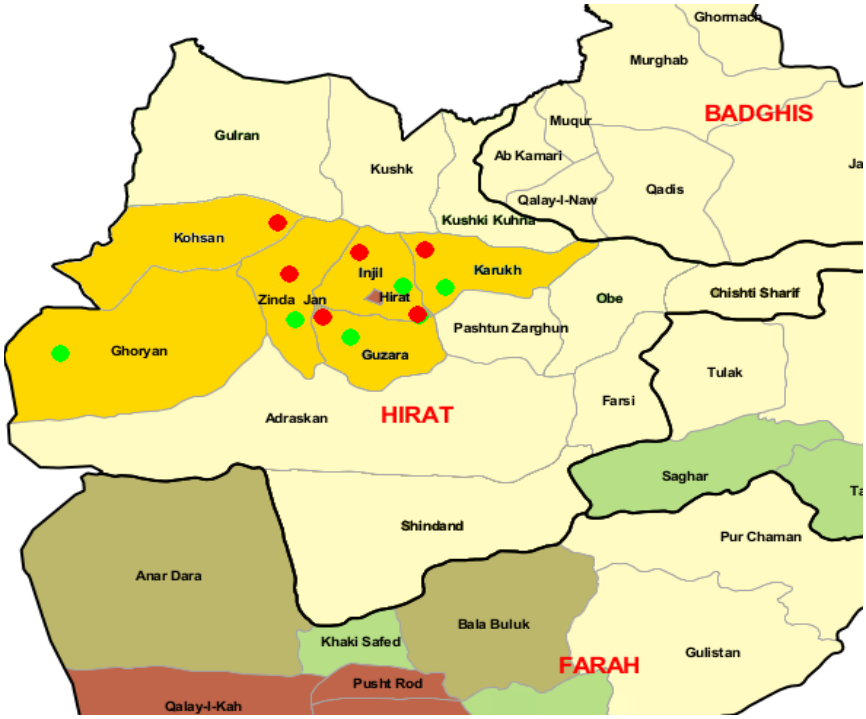


Figure 1. Map of Herat Province showing locations of study villages. Kuchi villages are shown as ●, and Sedentary villages as ●.

Figure 2. Meeting with village elders to explain the purpose of the study and the sampling procedures involved.

A total of 204 randomly selected households were sampled, and bloods were collected from 1,017 humans between 8 and 50 years of age, 877 goats, 1,155 sheep and 350 cattle. Blood samples were sent to the Provincial Veterinary Laboratory (PVL) in Herat for serum separation and storage in duplicate prior to transport to the veterinary and public health laboratories in Kabul for serological testing.

All animal sera were initially screened for *Brucella* antibodies using an AHVLA Scientific Rose Bengal Test (RBT) at the Central Veterinary and Diagnostic Research Laboratory (CVDRL). Samples were considered positive for *Brucella* antibodies if agglutination occurred after a period of mixing of four minutes. An AHVLA Scientific competitive ELISA (cELISA) was conducted on all samples that were RBT-positive and the tests were interpreted in series. It was decided to dispense with the RBT and only use the cELISA for human sera after the Central Public Health Laboratory (CPHL) found no RBT positive sera during preliminary testing of about 300 sera. All human and animal sera were then tested for Q fever at the CVDRL with a commercial indirect ELISAs; LSI™ LSIVET for animal and an IBL international two phase ELISA for human sera. Sera have been stored frozen at CVDRL and CPHL for CCHF testing at a future date.

Figure 3. A member of the field team records health and occupation data after collecting a blood sample from a village householder in Herat Province.

## Preliminary results

### Animals

Sera were collected from 1,143 blood samples from sheep, 876 from goats and 344 from cattle. The seroprevalence of brucellosis was 1.6% (95% CI=1–2.5) for sheep, 1.6 (95% CI=0.9–2.7) for goats, 0.29 (95% CI=0.02–1.8) for cattle and 1.4% (95% CI=1–2) overall.

*Brucella* seropositives were detected in 27 households and 10 villages. The prevalence in Kuchi villages was 2% (95% CI=1.3–3.1) and 0.8% (95% CI=0.4–1.5) in sedentary villages. The risk of abortion was higher in *Brucella* seropositive than in seronegative small ruminants; seroprevalences were higher in Kuchi than in Sedentary villages; and the risk of seropositivity increased with age.

Figure 4. Collecting blood samples from livestock in a semi-nomadic Kuchi village in Herat Province.

The seroprevalence of Q fever was much higher with 43.4% (95% CI=40.5–46.3) recorded for sheep, 52.7% (95% CI=49.4–56) for goats, 5.2% (95% CI=3.3–8.1) for cattle and 41.3% (95% CI=39.3–43.3) overall. Q fever seropositive animals were detected in all of the study villages and in 201 (98.5%) of the 204 study households. The respective prevalences for Kuchi and sedentary village animals were 44.3% (95% CI=41.4–47.2) and 38.5% (95% CI=35.9–41.3). Village level prevalences of brucellosis and Q fever in animals are shown in Figures 5 and 6.

### Humans

The overall seroprevalence of brucellosis in humans in the 11 study villages was 5.21, (95% CI=4–6.8) and seropositives were found in 10 villages. Humans with serological evidence of exposure to brucellosis were detected in 32 (15.7%) of the 204 study households and infection was strongly clustered at village level (Figure 7). Of the 53 *Brucella* seropositive humans, 47 were also seropositive for Q fever.

About 65% of the human participants were female and the 16 to 30 year old age group constituted the greatest proportion (42.4%) (Figure 7). The prevalences of *Brucella* and Q fever seropositives in individuals and in Kuchi and Sedentary villages are shown in Tables 1 & 2.

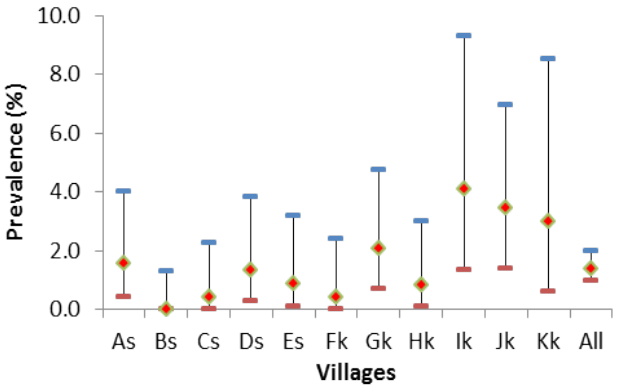


Figure 5. *Brucella* seroprevalences (◆) in animals with upper (▬) and lower (▬) 95 % CI for each study village (A–K). Sedentary villages are identified by (s) and Kuchi by (k).

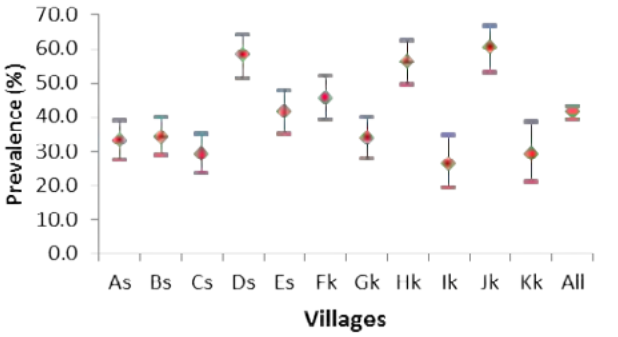


Figure 6. Q fever seroprevalences (◆) in animals with upper (▬) and lower (▬) 95 % CI for each study village (A–K). Sedentary villages are identified by (s) and Kuchi by (k).

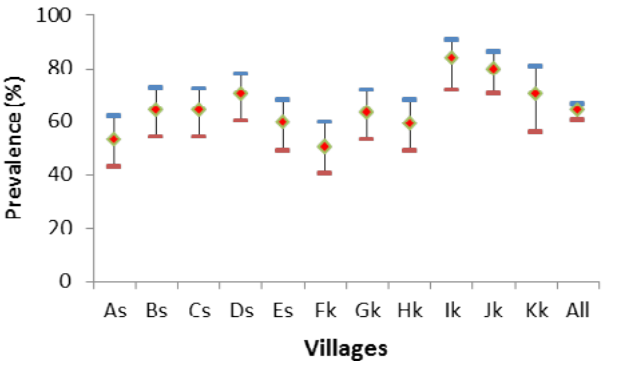


Figure 7. *Brucella* seroprevalences (◆) in people with upper (▬) and lower (▬) 95 % CI for each study village (A–K). Sedentary villages are identified by (s) and Kuchi by (k).

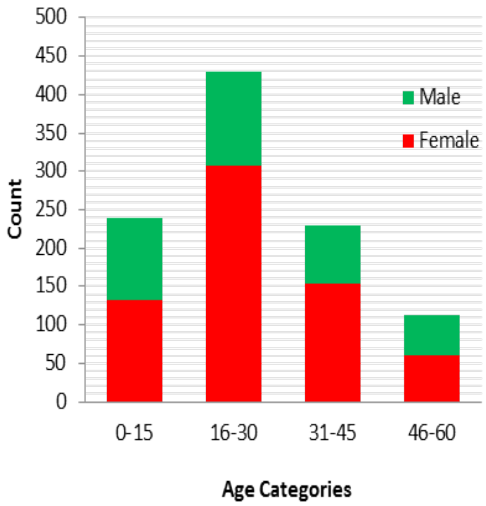


Figure 8. Gender and age distribution of human participants.

Bivariate analyses revealed significant associations between occupation, socio economic status, knowledge of brucellosis and the proportions of households with *Brucella* positive animals or abortions and *Brucella* positivity in humans. Analysis of the knowledge, attitudes and practices data showed that almost all household members had close contact with animals and many engaged in high risk activities such as slaughtering animals (20%), assisting at birth time (42%), shearing (58%), removing ticks (61%) and using unpasteurised milk (33%). About 50% of the household members who were interviewed were ill at the time of blood collection and about half of those about reported joint pain and fever and about 75% reported having body pain.

## Discussion

The One Health philosophy embodied in this study is thought to be a world first for investigation of brucellosis, Q fever and CCHF in the same human and animal population, which sets the scene for further collaborative studies of these zoonoses. The data are complex and analysis is on-going, but significant findings with relevance to human and animal health preventive programs have already emerged. The extremely high prevalences of serological evidence of exposure to Q fever in humans and animals was a surprising finding, while the prevalence of *Brucella* seropositivity was lower than expected.

Many infections with Q fever in people are asymptomatic, but a proportion of those who are acutely affected suffer from pneumonia and hepatitis. Endocarditis and a variety of debilitating symptoms are common manifestations of chronic infections. The mortality rate in the acute form is estimated to be 1 to 2%. The high prevalence of Q fever in young persons between 8 and 15 years of age reflects a high risk of exposure at an early age, which is particularly worrying.

The evidence of strong clustering of brucellosis at village level, and the higher prevalences in kuchi than in sedentary communities warrant further investigation to identify husbandry and management factors responsible for the high prevalences and to estimate the extent of wastage. All three diseases have common risk factors and the information from the KAP study will be highly relevant to awareness and disease prevention programs.

Table 1. Prevalence of seropositive people for brucellosis and Q fever according to gender.

| | Brucellosis | | | Q fever | | |
|---|---|---|---|---|---|---|
| Sex | N positive | N tested | Prevalence (95%CI) | N positive | N tested | Prevalence (95%CI) |
| Female | 27 | 656 | 4.1 (2.8–5.9) | 397 | 656 | 60.5 (56.7–64.2) |
| Male | 26 | 361 | 7.2 (5–10.3) | 253 | 361 | 70.1 (65.2–74.6) |
| Total | 53 | 1017 | 5.2, (4–6.8) | 650 | 1017 | 63.9 (60.9–66.8) |

Table 2. Prevalence of seropositive people for brucellosis and Q fever in Kuchi and Sedentary villages.

| | Brucellosis | | | Q fever | | |
|---|---|---|---|---|---|---|
| Village type | N positive | N tested | Prevalence (95%CI) | N positive | N tested | Prevalence (95%CI) |
| Sedentary | 17 | 512 | 3.3 (2.0–5.4) | 316 | 512 | 61.7 (57.4–65.8) |
| Kuchi | 36 | 505 | 7.1 (5.1–9.8) | 334 | 505 | 66.1 (61.9–70.1) |

## Lessons learned

A variety of challenges were encountered during the course of this study. The random selection process occasionally selected villages or households with insufficient numbers of livestock. This was overcome by having lists of reserve villages and households that had also been randomly selected. Occasionally, the survey team deviated from this plan and substituted a more cooperative neighbouring household instead.

Some difficulties were encountered with villagers not wishing to participate in the study, in these circumstances the Motivation Team revisited the villages and tried to secure their cooperation. If this failed another village was substituted.

The field team returned from the first few visits late in the day and had little time to process the samples. During later visits the team were better organised and faster but work often continued into the evenings in order to process the samples on the same day.

## Recommendations

Difficulties were more likely to arise if the survey team did not explain the objectives of the survey to the head of a selected household. It is recommended that permission to conduct the survey is gained from the head of the household before starting to collect samples or administering a questionnaire.

Supervision of the sampling teams is important, and those supervisors should cross-check the questionnaires from at least 1 or 2 households in each village.

When testing a large number of serum samples, a correct and well-organised serum storage system has to be used. Otherwise misplacement of serum samples will provide unreliable results.

## Acknowledgments

The study was jointly designed and coordinated by members the General Directorate of Animal Health and Production (MAIL) and Afghanistan National Public Health Institute (MoPH) working both in Kabul and Herat and local NGOs, HPRO and DCA-VET, the EU funded Animal Health

Development Program and by mentors from Massey University and the London School of Hygiene and Tropical Medicine.

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Figure 9. The Afghan One Health field team comprising human and animal health professionals.