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Working towards development of a sustainable brucellosis control programme, the Azerbaijan example

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A B S T R A C T

Brucellosis caused by *Brucella abortus* and *Brucella melitensis* is endemic in the Republic of Azerbaijan but a complex mix of fiscal, political and technical constraints has impeded regulatory authority decision making for adoption of a sustainable national control programme. This paper reports a series of epidemiologic studies of the disease in animals and humans which we conducted between 2009 and 2020. A preliminary study and a subsequent larger study using vaccination of all non-pregnant female sheep and goats of breeding age and all females between 3 and 8 months with conjunctival Rev1 vaccine both recorded significant reduction in small ruminant seroprevalences. A case control study of winter pasture flocks found many case and control farmers used raw milk to make dairy products for sale, ate fresh cheese and sold dairy products in unregulated markets. Almost all farmers expressed willingness to pay a portion of the costs associated with elimination of brucellosis from their flocks. A pilot human study in 2009 led to a large study in 2017 which recorded an overall seroprevalence of 8.1% in humans. Persons in farm related occupations were at greater risk than urban persons and males were more likely to be seropositive than females. Risk factors included keeping small ruminants, using raw milk cheese and slaughtering animals whereas having heard education information about brucellosis and vaccinating against brucellosis were protective.

1. Introduction

Brucellosis caused by *B. abortus* has been eliminated in most developed countries but remains a challenge for countries where *B. melitensis* also occurs (Hernandez-Mora et al., 2017; Nejad et al., 2020; Pappas et al., 2006; Racloz et al., 2013), despite availability of inexpensive and efficacious vaccines and easy to perform diagnostic tests (Abernethy et al., 2012; Nielsen, 2002). The purpose of this paper is to share our Azerbaijan experiences of controlling brucellosis in countries with low human and animal health resources and provide a perspective on issues that face veterinary and public health authorities for setting up sustainable disease control programmes. Azerbaijan is now classified as a country in transition from developing to developed status but developing is appropriate for much of the time since the mid-1990s when the activities described in this paper were performed.

The massive disarray following the collapse of the USSR and Soviet withdrawal in the early 1990’s was compounded by adsorption of about one million refugees from Azerbaijan territories occupied by Armenia. The post-Soviet independent Republic divided the large state controlled kolkhozes and sovkhozes collective farms into small parcels of land which were given, with livestock, to former members of the collectives and introduced the concept of privatisation of goods and services. The Soviet era system changed to free market with livestock owners owning their allocated small parcels of land and able to sell their farm products. However, fiscal constraints were severe and government veterinary services suffered from lack of funds with wages insufficient for living costs for the large contingent of veterinarians and veterinary technicians required to service the needs of a large number of small farms and carry

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out routine control programmes for priority diseases such as tuberculosis, brucellosis, sheep pox, anthrax, rabies and foot and mouth disease. The public and veterinary health authorities only just survived and started on a long and still ongoing journey of restructuring. In 1990 there were about 3000 employees in the State Veterinary Control Services (SVCS) and staff numbers have remained relatively stable over time. The Azerbaijan Food Safety Authority was established in late 2017 and was complemented by six zonal laboratories and a Central Veterinary Republican Laboratory (CVRL) for diagnostic services. In 2020 the Agency for Agricultural Services, Department of Animal Health and Veterinary Services had a chief veterinary officer and about 40 administrative staff, a research institute, 65 regional offices with a director and field staff, plus 47 diagnostic cabinets solely for Rose Bengal Serum Agglutination (RBT) testing for brucellosis. On-farm services were delivered by 798 veterinary field units staffed by 1624 veterinarians and veterinary technicians. Salaries were about €200 per month for veterinarians and €130 for veterinary technicians. Most field staff live in villages and each veterinarian or technician is responsible for providing animal health services to his own and one to two neighbouring villages and their widespread distribution throughout communities facilitates early detection of outbreaks of endemic and exotic disease. Government incomes are supplemented to some extent by an informal system of fees or payment in kind for clinical services for which most farmers are willing to pay. However, delivery of veterinary services is challenging in the Azerbaijan rural setting of about 740,000 small holdings with livestock and about 4300 villages.

Test and slaughter used for control of brucellosis in Soviet times when livestock were the property of the State has persisted with some modifications up to the present time. Brucellosis control was relatively easy in the collective farms but became far more logistically difficult to implement in the new setting of scattered livestock throughout numerous small holdings, compounded by shortages of vehicles for field staff, outdated equipment, little exposure to modern technology, and reluctance to slaughter animals without compensation.

Azerbaijan is a relatively small country of 86,600km^2 in the Caucasus with a population of about 10.4 million people. About half of the country is classified as mountainous and about 55% is agricultural land. The climate varies from semi-arid in the central part to sub-tropical and humid in the south-east littoral of the Caspian Sea. Large oil reserves are the major contributor to the economy and although agriculture only contributes 6% of GDP it employs about 38.3% of the workforce. Livestock production accounts for about 4.5% of Gross Domestic Product (GDP) and livestock population estimates for 2019 were about 2.6 million cattle and buffalo of which about 1.3 million were dairy cows and about 85,000 were dairy buffalo. The small ruminant population comprised about eight million sheep and about 620,000 goats. There are only about 5000 swine. Livestock numbers have been relatively stable since 2010 but milk production from cattle and sheep has increased by about 30%.

The activities reported in this paper were conducted as part of the World Bank (WB) funded Agricultural Credit and Development Project (ADSP) which started in 2000 with an objective of supporting animal health and veterinary services in the SVCS and promoting private veterinary services for improving production. It followed earlier WB funded initiatives conducted by the Food and Agriculture Organization, Rome, to set up private veterinary units (PVUs). By the end of 2012, 53 PVUs with 160 veterinarians in 55 districts had been established. The Agricultural Competitiveness Improvement Project (ACIP), which followed supported development and establishment of a national brucellosis control programme and this paper reports the programme's gradual development under the guidance of the Agricultural Program Implementation Unit (APIU), which was responsible for its implementation.

The SVCS brucellosis control programme in 2000 consisted of testing 50% of cattle and 20% of small ruminant populations, in all about 1 million tests, each year with the RBT and slaughter of seropositives. It was task-based rather than risk-based with scant regard to quality assurance for selection of animals for testing and performance of RBT and Complement Fixation (CFT) tests. Facilities were poor and many diagnostic cabinets and regional laboratories lacked cold storage for RBT antigen and used antigen that did not conform to OIE standards (Manual, 2018). Slaughter of infected animals was not compulsory and there was no compensation. The SVCS has persisted with this test and slaughter programme, which is based more on predefined targets rather than epidemiologic information.

Most sheep and goat livestock owners keep up to 15–20 animals, of which goats comprise about 1–2%. Village-based animals are grazed on common ground in the autumn and winter and may go to common mountain pastures for summer grazing. Large large winter pasture flocks which vary in size from several hundred to >1000 similarly move from their extensive lowland holdings to the mountain pastures. The grazing pattern is essentially transhumant with many small ruminants from villages and winter pasture flocks leaving lowland pastures in early summer for alpine pastures in the Major and Minor Caucasus Mountains and returning to the lowlands in early autumn. About 90% of animals are slaughtered in backyards or small privately owned slaughter points and there are few centrally located slaughter houses. Post-mortem inspection relies on visual appraisal by local SVCS staff at slaughter points and markets.

Disease information is recorded in paper based systems at each SVCS regional centre and entered into an Electronic Information Disease Surveillance System (EIDSS) developed by the United States Department of Defense and managed by a small epidemiology unit at SVCS headquarters in Baku.

Brucellosis is endemic in Azerbaijan and was an appropriate choice for Azerbaijan by the ACIP. It provided an opportunity to develop a modern science-based control programme and generate a better understanding of constraints to effective control of the disease in developing country settings.

2. Methods

This paper reports a series of cross-sectional epidemiologic studies of the disease in livestock and humans which were conducted between 2009 and 2020. The series started in 2009 with a pilot brucella seroprevalence study in livestock in four rayons, Balaken, Gakh, Sheki and Zagatal, and its efficacy was evaluated in 2015 when a seroprevalence study was conducted in 51 of 59 rayons in Azerbaijan. Another livestock seroprevalence study was conducted in early 2020 in 24 randomly selected rayons and one, Barda, which was purposively selected because of its high volume of animal trading. A two-stage sampling design was used for all livestock serosurveys with random selection of five villages in each rayon followed by random selection of 120 female small ruminants and 120 female large ruminants (cattle and buffalo) of breeding age from systematic randomly selected households in villages and from 100 randomly selected small ruminants from randomly selected winter pasture flocks. The sampling strategy for selected households was to sample all female goats and sheep up to a combined maximum of five small ruminants per household and keep visiting households until the required number of samples was taken. In circumstances where a village did not have enough animals, the sampling team proceeded to the next closest village to collect the balance.

An outcome of the 2015 study was a case control study which used data from winter pasture flocks to identify management factors associated with brucellosis infection in winter pasture flocks. As part of the 2015 study five winter pasture flocks had been randomly selected from lists of winter pasture flocks in each of 43 rayons where there were winter pasture flocks. A retrospective case control study of 88 flocks with no evidence of infection (controls) and 108 flocks in which seropositives were found (cases) was conducted by personal interviews with flock managers in early 2017.

The 2009 pilot livestock study was complemented by a pilot human study in February 2011 in pilot study rayons, Zagatal and Balaken. The study targeted households with seropositive animals, local market milk product vendors, veterinarians and their wives. The objectives of the study were to determine brucella seroprevalences in the target population, provide training for public health workers in detection and diagnosis of brucellosis, improve collaboration between SVCS and the Ministry of Health (MOH) for dealing with zoonoses, and raise community awareness about brucellosis. Brucellosis at that time was not considered a priority disease by the MOH. The sampling strategy was biased towards high risk groups which included farmers, farm workers and veterinarians and was not representative of the wider community. A Knowledge, Attitudes, Practices (KAP) study, not reported here, was also conducted among participants.

A second cross-sectional human seroprevalence study and a KAP study (see supplementary material) commenced in September 2017 and was completed in February 2018. Randomly selected participants (11,270) came from 190 villages and towns, which were randomly selected from 38 rayons, and from four regional cities, Naftalan, Gəncə, Şirvan and Sumqayit. Numbers of participants were 110 for each of four regional cities, 60 for each of 38 regional towns and 45 for each of 190 villages. All sera were first tested at the Republican Antiplague Station with the RBT and positive sera were then tested with *Brucella* M (immunoglobulin IgM) and immunoglobulin G (IgG) enzyme immunoassays. Sera were considered positive if they were test positive to both the RBT (1021 positive) and either one or both immunoassays (908 positive). Questionnaire data for the KAP study were collected from participants at the time of blood collection.

2.1. Data recording and conduct

Numbers of female large and small ruminants of breeding age owned by each household and the species, age and Rev1 vaccination status of the animals sampled were recorded at the time of sampling. In all surveys, blood samples were collected in plain sterile vacuum tubes, individually identified by rayon, village, species and sequence in each household. Blood samples were collected in plain sterile vacuum tubes and transported to regional veterinary laboratories for processing and testing with the RBT. Sera with a positive or suspicious RBT result were tested at the CVRL in Baku with a competitive ELISA (cELISA). Animals were considered test positive if they tested positive to the RBT and the cELISA. All data were stored in a Microsoft Access database. EpiInfo 7.1.2.0 was used for questionnaire design and data entry for the case control study and the 2017 human studies.

2.2. Statistical analysis

An Access database was used for recording all animal test data and EpiInfo 7.1.2.0 was used for construction of questionnaires and recording of human study data. EpiInfo 7.1.2.0. and OpenEpi.com were used for descriptive statistics (prevalence estimates, Risk Ratios (RR) and Odds Ratios (OR) and examining data which could conceivably be associated with outcomes of interest as a first screening process for multivariable analyses. Variables significant at $p < 0.10$ in the bivariate analyses were entered into separate multivariable mixed logistic regression models built with random effect for rayon to evaluate factors associated with winter pasture farm disease status in the winter pasture case control study, and random effect for location type for factors associated with occupation, gender and urban or rural location, and factors associated with effects of farming, food preparation, hygiene and occupation on seropositivity in the 2017 human study.

Statistical analyses were performed using EpiInfo version 7.1.2.0, and Stata 13 (StataCorp LP). Graphics were constructed in QGIS 3.10 and Microsoft Excel. Unless stated otherwise, prevalences are reported throughout the paper as percentages with 95% confidence Intervals (CI) in brackets.

2.3. Ethics statement

The human study was approved by the Ministry of Health's Republican Anti-Plague Station, Baku and all blood samples were taken by qualified medical staff. Ethics approval for sampling of the animals for this study was not required for taking blood because it is a normal husbandry procedure and was supervised or conducted by veterinarians. This is in line with Animal Welfare legislation in New Zealand and current practice in the Ministry of Agriculture in Azerbaijan.

2.4. Livestock studies

2.4.1. Pilot study methods

The pilot study was designed to evaluate the efficacy of vaccination of small ruminants (sheep and goats) with Rev1 vaccine. It was conducted in four selected rayons, Balaken, Gakh, Sheki and Zagatala with sample collection in 2009 and in 2015. It aimed to vaccinate all non-pregnant female small ruminants of breeding age with quality assured conjunctival Rev1 in the first two years of the study and all females between 3 and 8 months in each year and identify all vaccinates with an ear notch. Vaccination was twice yearly to account for year-round breeding and to reduce the risk of abortions (Jimenez de Bagues et al., 1989) from vaccination of pregnant animals. Vaccines were tested for the induction of antibodies after arrival in about 20 serologically negative sheep and in animals from several randomly selected flocks about 3 weeks after vaccination.

2.4.2. Pilot study results

A total of 1342 of 11,917 individual livestock holdings and 20 winter pasture flocks were sampled, three from Gakh, 11 from Zagatala and six from Sheki. There were no winter pasture flocks in Balaken. Numbers of small ruminants and cattle and buffalo tested and the apparent prevalences with 95% confidence intervals were calculated for each group. The overall prevalence in cattle was 2.2% and in small ruminants was 3.3% in the four pilot rayons. However, high prevalences of 4, 11.8 and 4.1% were found in randomly selected winter pasture flocks in Gakh, Sheki and Zagatal. Test positive animals were found in 18 of the 20 villages sampled with test positive sheep in seven and test positive cattle in 17. None of 106 village-based goats was seropositive. Seropositive buffalo were found in 2 of the 14 villages in which buffalo were tested but few buffalo were tested in each village and only 130 buffalo were tested In total. The 2015 survey conducted in 51 rayons used the same sampling strategy as the 2009 pilot study. Prevalences in small ruminants in 2015 were significantly lower than in 2009 (Risk Ratio 0.04 (0.0, 0.1) and there was a smaller reduction in cattle prevalences (Risk Ratio 0.57 (0. 0.9). The 2009 and 2015 prevalences with 95% CI error bars for each of the four pilot study rayons are shown in Figs. 1 and 2.

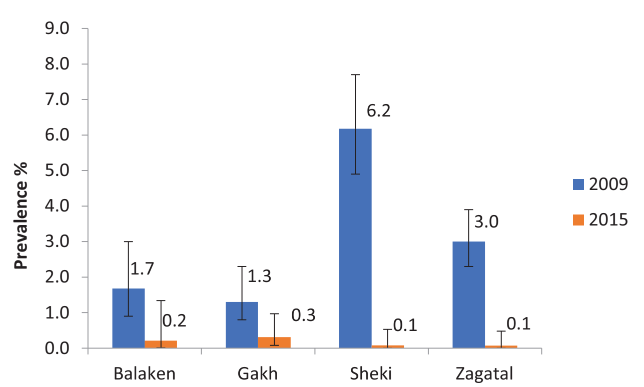

Fig. 1. Histogram showing 2009 and 2015 apparent prevalences with 95% confidence interval error bars for test positive small ruminants in village-based and winter pasture flocks in the four pilot study rayons.

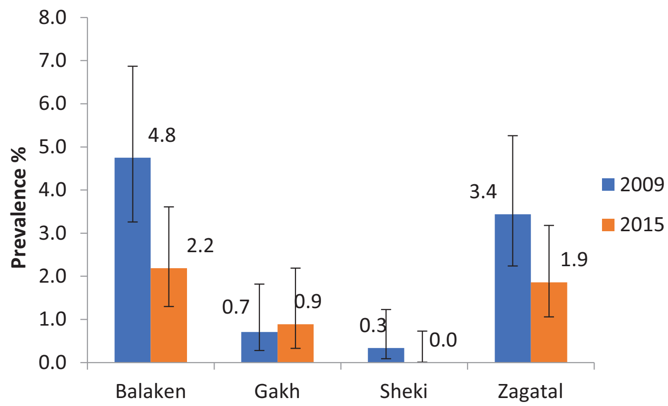

Fig. 2. Histogram showing apparent 2009 and 2015 prevalences with 95% confidence interval error bars for test positive cattle in in the four pilot study rayons.

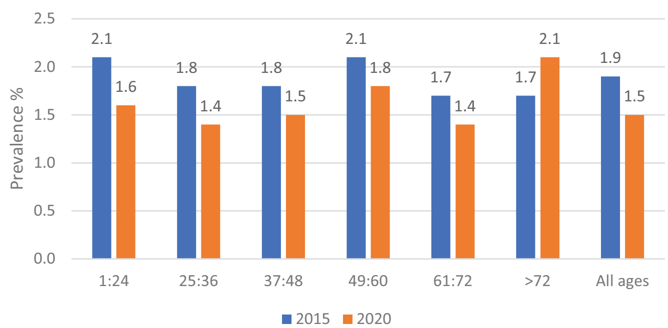

Fig. 3. Comparison of seroprevalences by age groups for small ruminants in the 2015 and 2020 serosurveys.

2.5. Comparisons between 2009, 2015 and 2020 livestock seroprevalence studies

Gakh and Zagatala were sampled in all three serosurveys. Significant reductions were recorded for all species and small ruminants between 2009 and 2020 but only a slight reduction for cattle in Gakh and no difference for cattle in Zagatala.

Comparison of the 2015 survey prevalences for all 51 rayons with the 2015 prevalences for the one purposively selected and 24 randomly selected in 2020 showed all categories except for winter pasture flocks were similar with overlapping 95% confidence intervals. The 2020 winter pasture prevalence for 25 rayons was slightly lower at 1.9% (1.6, 2.2) than the estimate of 2.5% (2.3, 2.7) for the 51 rayons which were sampled in 2015. These results gave confidence in the 2020 serosurvey sampling strategy.

The overall RR for all species in a comparison of the 2015 and 2020 surveys in 25 rayons was 0.8% (0.7, 0.9) (Table 1). Differences between overall prevalences in individual rayons in both surveys were small in most cases and may have been due to sampling variability in selection of livestock owners at different locations and variability in prevalences and proportions of species of livestock. Small ruminant prevalences were low in both surveys (Table 1) with a small but significant difference between 2015 and 2020, Risk Ratio 0.8 (0.7, 0.9). Village-based and winter pasture small ruminant seroprevalences were lower in 2020 than in 2015 (Table 1). Goats in 2020 were more likely to be positive than sheep, Risk Ratio 1.4 (1.1, 1.8), and small ruminants in winter pasture flocks were more likely to be test positive than village-based small ruminants, Risk Ratio 1.7 (1.5, 2.0).

Seroprevalences of 1.9% (1.6, 2.2) and 2.5% (1.4, 4.42) were recorded for winter pasture sheep (9609) and goats (440) in 2020. The

Table 1
Comparison of prevalences with 95% confidence intervals for all species, sheep, goats, small ruminants, cattle and buffalo, and winter pasture and village-based small ruminants for the 2020 survey with those from the same rayons in the 2015 survey.

Species	2015 survey (25 rayons)		2020 survey (25 rayons)		Risk ratio
	N tests	Prevalence %	N tests	Prevalence %	
All species	45,571	1.6 (1.5, 1.7)	41,419	1.3 (1.2, 1.4)	0.8, 0.7, 0.9)
Sheep	28,170	2.0 (1.8, 2.)	29,671	1.5 (1.4, 1.7)	0.8 (0.7, 0.9)
Goats	1119	1.5 (0.8, 2.2)	1188	1.9 (1.3, 2.9)	1.3 (0.7, 2.4)
Sheep&goats	29,289	1.9 (1.8, 2.1)	30,859	1.5 (1.4, 1.7)	0.8 (0.7, 0.9)
Cattle	16,205	2.0 (0.8, 1.1)	10,511	0.8 (0.6, 0.9)	0.8 (0.6, 1.03
Buffalo	77	0	49	0 (0, 7.3)	
Cattle&buffalo	16,282	1.0 (0.8, 1.1)	10,560	0.8 (0.6, 0.9)	0.8 (0.6, 1.0)
Village livestock	34,908	1.3 (1.2, 1.4)	31,370	1.2 (1.0, 1.3)	0.8 (0.7, 1.5)
Winter pasture	10,663	2.6 (2.3, 2.9	10,049	1.9 (1.6, 2.2)	0.7 (0.6, 0.9)
Village SR	18,869	1.6 (1.4, 1.7)	20,810	1.4 (1.2, 1.5)	0.9 (0.7, 1.0)
Winter past SR	10,420	2.6 (2.3, 3.0)	10,049	1.9 (1.6, 2.2)	0.7 (0.6, 0.9)

SR = small ruminants.
Differences between the 2015 and 2020 small ruminant age group prevalences were small (Fig. 3). Risk Ratio 0.8 (0.7, 0.9). However, there were consistent reductions in the 2020 age groups from 1:24 months to 49:60 months which would not have been represented in the 2015 serosurvey, Risk Ratio (0.85 (0.72, 1.0), unlike small ruminants >60 months which were represented.

winter flock prevalences classified by age groups were similar for age groups between 37 and > 72 months but were lower in 2020 for ages up to 36 months and for overall ages (Fig. 4).

Seroprevalences of 1.4% (1.2, 1.5) and 1.6% (0.9, 2.8) were recorded in 2020 for village-based sheep ($n = 20{,}062$) and goats ($n = 748$). Village small ruminant prevalences recorded in 2015 and 2020 were similar for most age groups (Fig. 5). Small ruminants prevalences in village-based and winter pasture flocks in 2020 were 1.4%(1.2, 1.5) and 1.9% (1.6, 2.2) in contrast with 2015 where the respective prevalences were 1.6% (1.4, 1.7) and 2.6% (2.3, 2.9) (Table 1 and Fig. 5).

Cattle and buffalo seroprevalences were lower in 2020 than in 2015 with 1.0% (0.8, 1.1) and 0.8% (0.6, 0.9) in 2015 and 2020 and Risk Ratio 0.8 (0.6, 1.0). A feature of the 2020 cattle test results was the clustering of seropositives in relatively very few households. As an example, only 12 of about 278 Agsu rayon households and nine of about

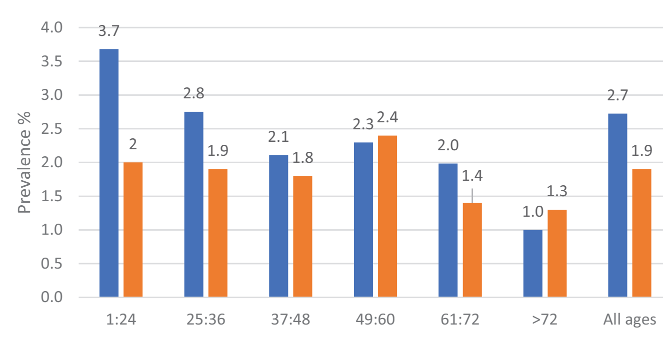

Fig. 4. Comparison of seroprevalences by age groups for winter pasture flocks in the 2015 and 2020 serosurveys.

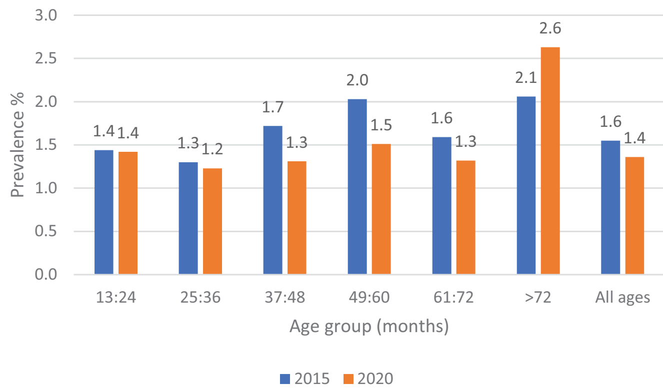

Fig. 5. Comparison of seroprevalences for village-based small ruminants in the 2015 and 2020 serosurveys.

138 Agcabedi rayon households had positive cattle.

The spatial distributions of small ruminants prevalences in rayons in 2015 and 2020 are shown in Figs. 6 and 7 and for cattle in Figs. 8 and 9. Individual rayon prevalences in winter pasture flocks varied from 0 to 14.3%, mean 2.7, median 1.7 in 2015 and from 0 to 8.2%, mean 1.7, median 0.8 in 2020. Similar statistics for small ruminants were 0 to 4.7%, mean 1.4, median 0.7 in 2015 and 0 to 4.8%, mean 1.3, median 0.7 in 2020 and for cattle 0 to 3.7%, mean 0.9, median 0.7 in 2015 and 0 to 3.3%, mean 0.7, median 0.5 in 2020.

2.6. Winter pasture flock case control study

Results from bivariate analyses indicated lower risk for flocks which migrated than those which stayed at home, OR 0.3 (0.2, 0.6), higher risks for changing the makeup of breeds in their flocks, OR 3.0 (1.7, 5.5), buying breeding animals from markets, OR 3.0 (1.7, 5.3), having contact with other flocks in the summer pastures, OR 4.4 (2.2, 8.5) and disposal of placentas by feeding to dogs, OR 6.4 (3.4, 12.1) as opposed to disposal by burying, OR 0.2 (0.1, 0.4). Case farms were less likely than controls to consider brucellosis to be a problem, OR 0.2 (0.1, 0.5) and less likely than controls to have separate stalls for birthing, OR 0.2 (0.07, 0.4). Case farmers were less likely than control farmers to think that people prefer to purchase brucellosis-free dairy products, OR 0.4 (0.2, 0.9) and less likely than control farmers to think that people would be willing to pay more for these products, OR 0.6 (0.3, 1.0). Almost all case (98 of 108) and control farmers (80 of 88) said they were willing to pay a portion of the costs associated with elimination of brucellosis in their flocks.

A mixed effect logistic regression with rayon entered as a random effect indicated that livestock in flocks which went to winter pastures were less likely to be infected than livestock in flocks which stayed at home, farmers who changed breeds of sheep were more likely to be infected than farmers who had not changed breeds, farmers who had brucellosis in their flocks were less likely than farmers with no infection to think that brucellosis is a problem and feeding placentas to dogs was a risky practice (Table 2).

Other findings of special interest included finding 38 case and 39 control farms used raw milk to make cheese, eight case and four control farms used raw milk to make fermented milk, 16 case farmers and 14 control famers ate fresh cheese and 38 case and 41 control farms sold cheese in villages or in local markets. Whey left over from cheese making was fed to cats and dogs by 50 farmers and to sheep and cattle by 19.

3. Human studies

Blood samples taken from 278 females and 299 males were ruled to have serological evidence of exposure to brucellosis after a positive RBT

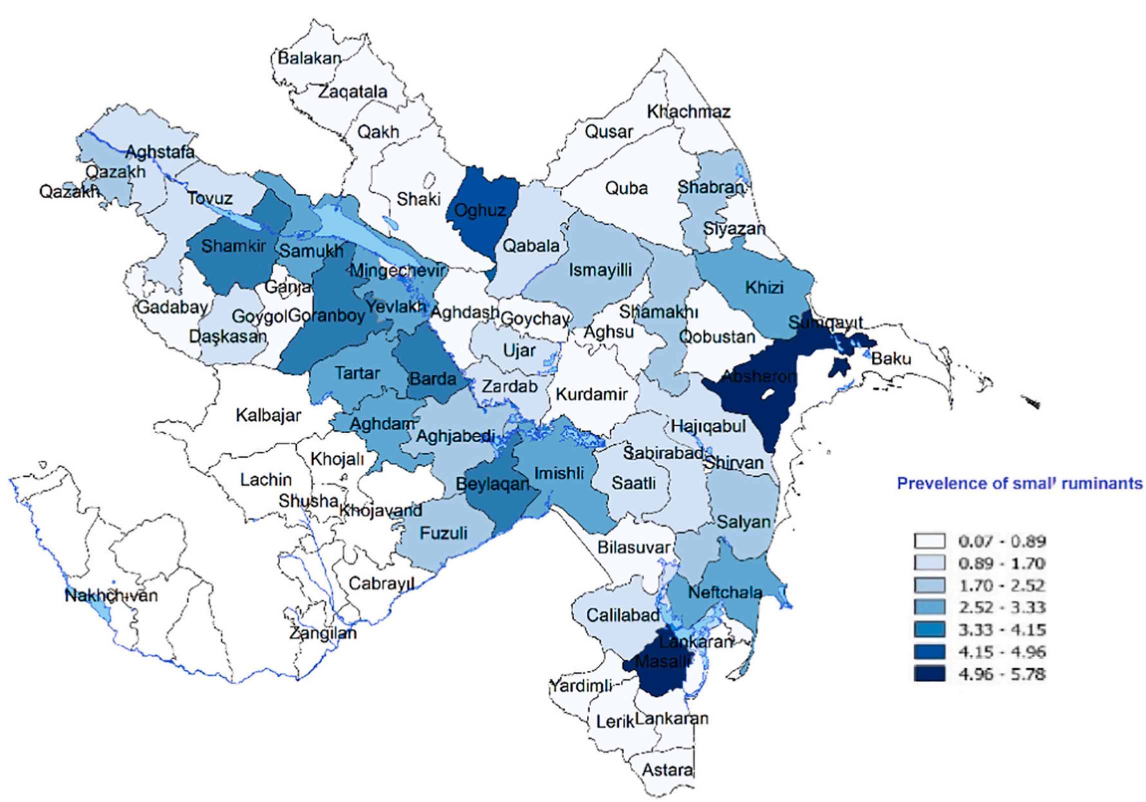

Fig. 6. Spatial distribution of prevalences of test positive small ruminants among 51 rayons (Abseron, Agcabadi, Agdam, Agstafa, Agsu, Astara, Barda, Balakan, Beylagan, Bilusivar, Calilabad, Dashkasan, Fuzuli, Gadabay, Goranboy, Goycay, Goygol, Haciqabul, Imisli, Ismayilli, Lankaran, Lerik, Kurdamir, Masalli, Oguz, Qax, Qazak, Qabala, Qobustan, Quba, Qusar, Saatli, Sabirabad, Sabran, Salyan, Samaxi, Sheki, Samkir, Samux, Siazan, Yartar, Tovuz, Ucar, Xacmas, Xizi, Yardimli, Yevlax, Zaqatala, Zardab) sampled in Azerbaijan in 2015.

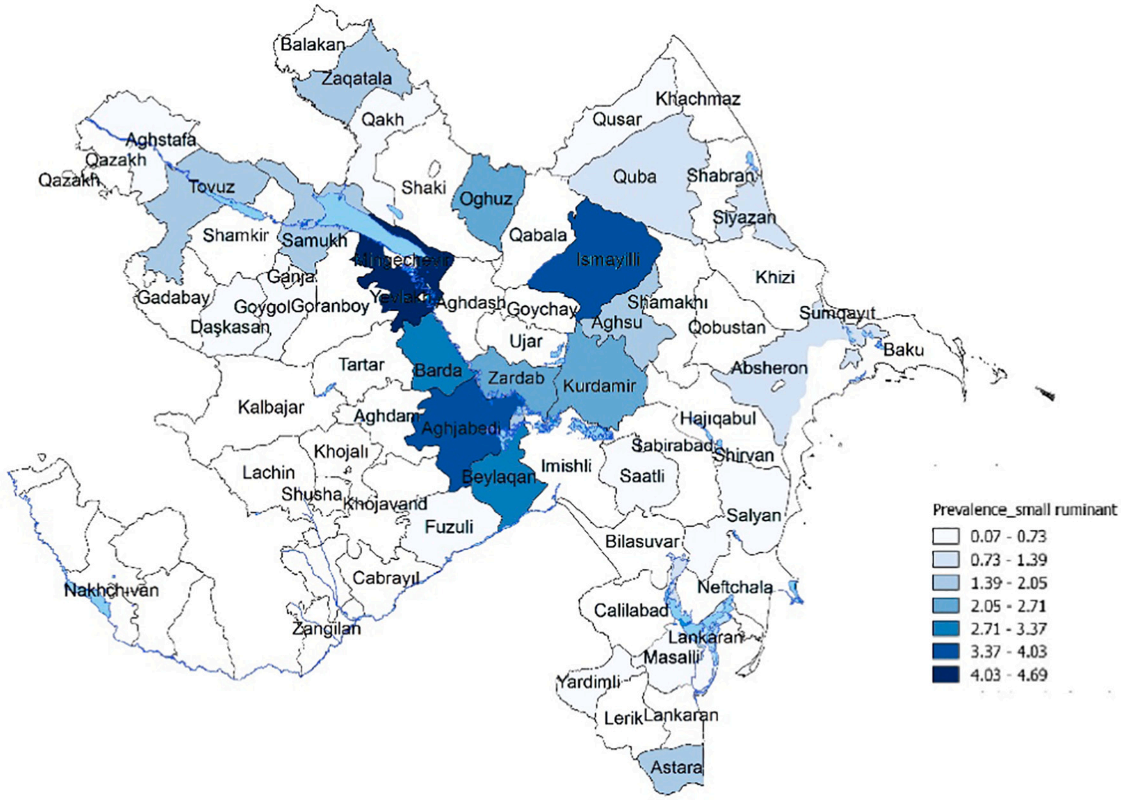

Fig. 7. Spatial distribution of prevalences of test positive small ruminants among 25 rayons (Abseron, Agcabadi, Agstafa, Agsu, Astara, Barda, Beylagan, Dashkasan, Goygol, Ismayilli, Kurdamir, Masalli, Oguz, Qax, Quba, Qusar, Saatli, Salyan, Samux, Siazan, Tovuz, Yardimli, Yevlax, Zaqatala, Zardab) sampled in Azerbaijan in 2020.

and a Standard Agglutination Test (SAT) with a titre >1:40. Males were more likely to be test positive than females, Risk Ratio (5.3 1.4, 3.6). One of 43 persons (2.3%) in the 10:19 age group was seropositive and the prevalence in persons >20 years old was 7.8%. Fourteen (23%) of 59 farmers and nine (16%) of 56 veterinarians were test positive. Seven (37%) of 19 persons who drank raw milk tested positive but only 33 (8.1%) of 408 who did not drink raw milk were test positive, Risk Ratio 4.6 (2.3, 8.9).

Overall seroprevalences were similar for the three locations (Table 3) but there was considerable variation within locations. There were 1, 3, 11 and 10 seropositives among 110 participants in each of the regional cities, Naftalan, Gəncə, Şirvan and Sumqayit. Numbers of seropositive persons in regional towns varied from 1 to 27 about a mean of 5.6.

Seroprevalences were similar for all age groups, except for the all-student 10:14 age group in which three of 128 were seropositive. There were 37 seropositives in the 15:19 age group of 328 persons, indicating exposure to infection at a relatively young age. Table 5 reports results from simple logistic regression which was used to identify occupations with ORs >1 and therefore considered to be high risk5.

A mixed effects logistic regression model was constructed to show the relative effects of farm related occupations, gender and village location (Table 6).

Of the 2362 participants with farms, 2116 (89.6%) kept cattle, 1595 (67.5%) had small ruminants and 1445 (61.2%) had both cattle and small ruminants. Persons with farms were more likely to be test positive, OR 1.3 (1.2, 1.6) than persons not owning a farm. Odds Ratios for testing positive among participants who kept cattle were 1.4 (0.8, 2.2), and 3.6 (2.4, 5.3) for those keeping small ruminants, and 1.8 (1.1, 2.9 for those keeping both cattle and small ruminants. Data from KAP questions related to farming practices, care of animals, slaughtering and hygiene, and use of dairy products were used to construct a mixed effects logistic regression model for effects of farming, food practices, hygiene and occupation (Table 7).

The KAP study recorded information about the use of dairy products and meat among the 2362 participants with farms. Those making dairy products (285) or making cheese from raw milk (143) were at greater risk of testing positive, OR 3.3 (2.4, 4.5) and 6.3 (4.2, 9.0).

Raw milk consumption was reported by 199 (18%) of 11,270 participants; OR for testing positive 5.7 (4.2, 7.8), and fresh cheese by 1280 (11,4%); OR for testing positive 61.1 (51.1, 73.0). The main sources of information about brucellosis reported for the 4800 participants who had heard about brucellosis were doctors (60.4%), TV (33.9%), veterinarians (33.4%), newspapers (8.3%) and school (4.1%). The OR for testing positive for persons who had heard about brucellosis was 0.3 (0.3, 0.4).

4. Discussion

The pilot study programme, conducted between 2009 and 2015 in Balaken, Gakh, Sheki and Zagatala rayons, of annual vaccination with conjunctival administered Rev1 to all female small ruminants between three and eight months old and all non-pregnant small ruminants of breeding age in the first two years provided support for adoption of a national control programme using the same vaccination strategy. Significant reductions in seroprevalences were recorded for village-based and winter pasture small ruminants between 2009 and 2015 and in the two rayons which were sampled again in the 2020 serosurvey. The nationwide control programme for small ruminants using the same methods started in Spring 2016 and 6.35 million small ruminants had been vaccinated by the end of March 2017 and 11.4 million doses had been administered by the end of 2019. Vaccination with Rev1 vaccines had been sporadic before 2015, vaccines did not comply with OIE standards (OIE, 2008) and cold chain was not always maintained. The main thrust of the SVCS programme was annual test and slaughter of

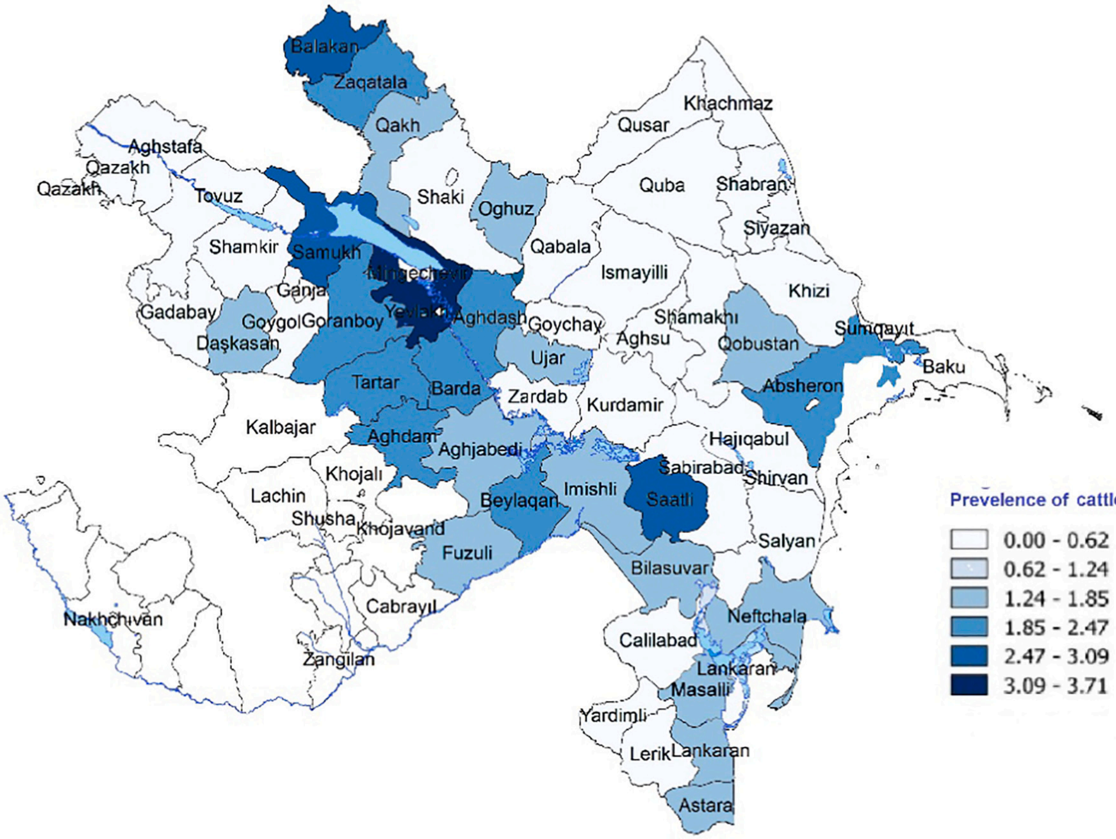

Fig. 8. Spatial distribution of prevalences of test positive cattle among 51 rayons sampled in Azerbaijan in 2015.

50% of cattle and buffalo and 25% of small ruminants but quality assurance for testing was poor and SVCS 2015 test results of 0.1 for cattle and 0.2% for small ruminants contrasted with the 1.8 and 0.8% for small ruminants and cattle reported by the 2015 serosurvey supervised by the APIU. An important finding from all APIU supervised serosurveys was relatively high seroprevalences in winter pasture flocks, one with 43% and six >10% in 2015. The transhumant nature of many of these enterprises meant that they were not easily recognized by the SVCS and as a result had been largely ignored.

Decisions about vaccination of cattle were deferred until uncertainties about which species of brucella were involved and vaccine efficacy of Strain 19 for control of *B. melitensis* could be resolved and was further complicated by widespread promotion of RB51 vaccine. Cultures of material from seropositive cattle were shown to be due to both species of Brucellae in 2012 (Galib Abdulaliev pers. com) but vaccination of female calves 3 to 8 months of age with conjunctival administered Strain 19 did not commence until its evidence of its efficacy in cattle was reported in 2016 (van Straten et al., 2016). The programme started with vaccination of female calves 3 to 8 months of age in 2017 and expanded in 2020 to include vaccination of adult non-pregnant females after finding clustering of infection in some cattle enterprises producing milk for sale.

The reduction for small ruminants from 1.9to 1.5% between 2015 and 2020 was greatest in animals <60 months of age which would not have been represented in the 2015 survey and contrasts with the no differences in >60 month animals which were represented in the 2015 survey and for which a reduction in prevalence would not be expected. A reasonable conclusion is that the reduction in the <60 month animals can be attributed to vaccination of adult and immature females with conjunctival Rev1. Not enough time has elapsed since the introduction of vaccination of cattle with Strain 19 by the conjunctival method in cattle to show any effect from vaccination which started in 2017. Clustering of infection was evident in all surveys, and especially in the winter pasture flocks where vaccination coverage has been lower than in village flocks and where some very high prevalences were recorded. Higher coverage of vaccination in all locations, ideally with slaughter of infected animals, will be required to reduce the incidence of infection in animals and in people who are in direct contact with livestock and indirect contact through consumption of dairy products (Abedi et al., 2020; Cooper, 1992).

Higher prevalences in winter pasture small ruminants than in village-based small ruminants were consistent findings in all serosurveys. It was an important finding for the MOH, given the role of winter pasture flocks in producing soft cheeses for human consumption, and for the SVCS for targeting winter pasture flocks for special attention. Serosurvey data, not shown here and based on farmer recall, indicated low coverage of vaccination was associated with higher risk of infection in unvaccinated animals but the data may have been biased by persons in charge of animals at the time of sampling not knowing vaccination histories. Observations by project staff during vaccination programmes and at survey recording for evidence of previous vaccination with Rev1 have consistently shown that compliance with ear notching was good in some rayons and poor in others. Furthermore, there were problems with its application when other owner identification ear notches were present and with no ears in one particular breed of sheep. Experience has clearly shown that ear notching, despite ease of application and very low cost, is not an effective method and should be replaced with mandatory identification of vaccinates with an approved ear tag.

Very low prevalences were reported for some villages and rayons, e. g. Gakh, and regulatory authorities are now considering introducing confirmatory testing of RBT test positives with an approved ELISA to reduce the risk of slaughtering RBT test positive non-infected animals in those locations. The issue is more serious for cattle than for small ruminants because of their higher value and livestock owners not wanting

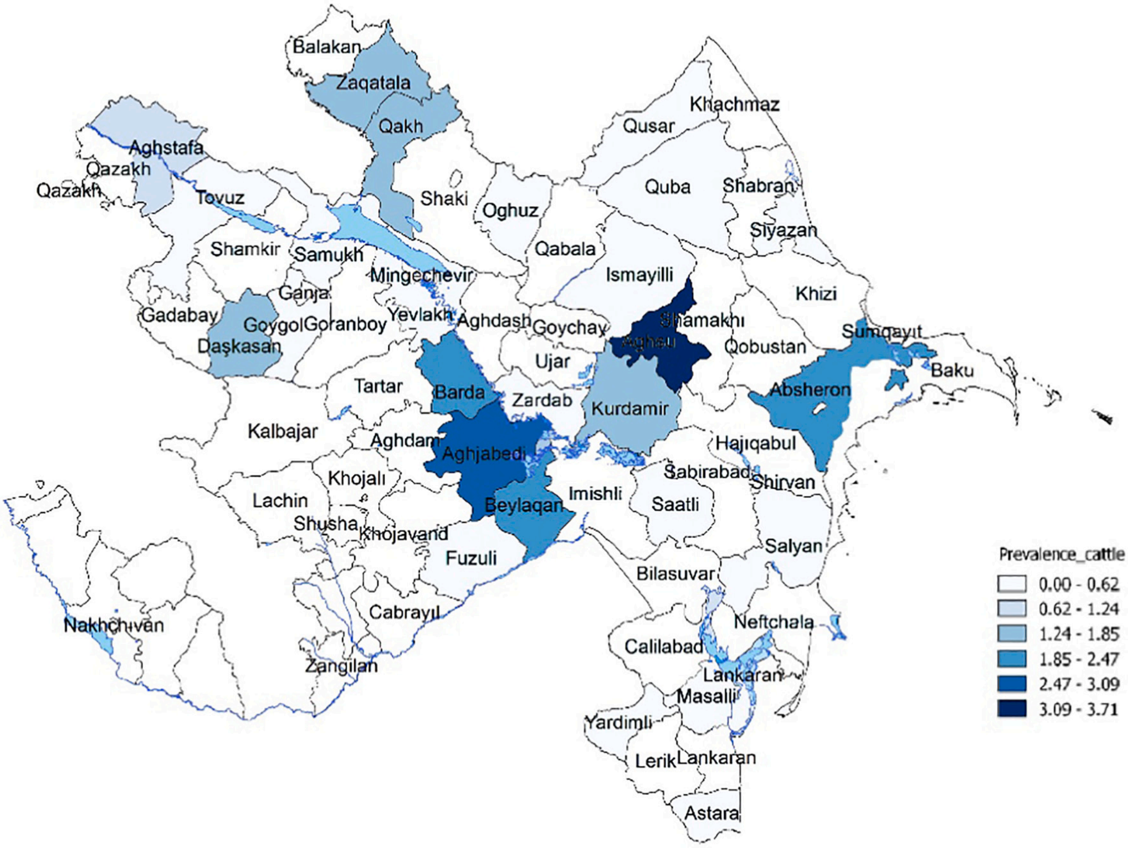

Fig. 9. Spatial distribution of prevalences of test positive cattle among 25 rayons sampled in Azerbaijan in 2020.

Table 2
Mixed-effects logistic regression results for *Brucella* seropositivity in small ruminants with rayon incorporated as a random effect.

Predictor variables	Level (number)	OR (95% CI)	*P* value
Staying at home and not migrating	Yes (122)	0.2 (0.1, 0.5)	0.002
	Migrating (74)	Reference	
Changing breeds	Yes (100)	2.5 (1.1, 5.89)	<0.04
	Not changing (88)	Reference	
Brucellosis considered a problem	Yes (148)	0.1 (0.2, 0.3)	<0.001
	Not a problem (48)	Reference	
Feeding placentas to dogs	Yes (115)	7.0 (2.7, 18.2)	<0.001
	Not feeding to dogs (81)	Reference	

Table 3
Numbers of human tests conducted, numbers positive and seroprevalences with 95% confidence intervals in brackets for 4 regional cities, 60 regional towns and 190 regional villages in Azerbaijan.

Locations (number in brackets	N tests	N positive	Seroprevalence % (95% CI)
Regional cities (4)	440	25	5.7 (3.9, 8.3)
Regional towns (38)	2280	238	10.4 (9.2, 11.8)
Regional centres[a] (42)	2720	263	9.7 (8.6, 10.8)
Regional villages (190)	8550	666	7.8 (7.2, 8.4)
Overall total	11,270	929	8.2 (7.8, 8.8)

Seroprevalences were similar for females over all locations and higher for males in regional centres but not in villages or overall locations (Table 4).

[a] Regional centres includes regional cities and towns.

Table 4
Table showing the number of test positive male and female persons with number tested for each location in Azerbaijan and the prevalence percentages with 95% confidence intervals in brackets for males and females in each location.

Location	Male participants		Female participants	
	N tests	Prevalence% (95% CI)	N tests	Prevalence% (95% CI)
Regional centres	775	11.5 (9.4, 13.9)	1945	7.9 (6.8, 9.2)
Regional villages	4180	8.4 (7.6, 9.3)	4370	7.2 (6.5, 8.0)
Regional total	4955	8.9 (8.1, 9.7)	6315	7.4 (6.8, 8.1)
Overall total	5348	10.1 (9.3, 10.9)	7242	9.0 (8.4, 9.7)

Table 5
High risk occupations for brucellosis in Azerbaijan, numbers sampled, numbers of seropositives and Odds Ratios >1.0 with 95% CIs in brackets for Odds of seropositivity.

High risk occupations	N sampled	N seropositive	Odds Ratio (95% CI)
Butcher	42	13	5.2 (2.7, 10.0)
Farmer	569	83	2.0 (1.6, 2.6)
Housewife	2082	224	1.5 (1.3, 1.8)
Meat seller	55	11	2.9 (1.5, 5.6)
Nurse	584	59	1.3 (1.0, 1.7)
Shepherd	50	13	4.1 (2.2, 7.7)
Working	165	22	1.8 (1.1, 2.8)
Milk seller	16	3	2.6 (0.8, 9.3)

to slaughter non-infected valuable animals. The case control study, using positive flocks as cases and negative flocks as controls, was an important outcome of the 2015 survey. It identified farm practices

Table 6
Odds Ratios with 95% confidence intervals in brackets from a mixed effects model with location entered as a random effect for effects of farm and urban occupations, gender and location in village or regional centres on seropositivity to brucellosis in Azerbaijan.

Predictor variable	Level (number)	Odds Ratio (95% CI)	*P* value
Farm related occupation	Yes (2822)	2.2 (1.9, 2.6)	<0.001
	Urban occupation (8448)	Reference	
Gender	Male (4955)	1.5 (1.3, 1.8)	<0.001
	Female (6315)	Reference	
Village location	Village location (8550)	0.3 (0.2, 0.3)	0.01
	Regional centre (2720)	Reference	

Table 7
Estimates of Odds Ratios with 95% confidence intervals in brackets from a mixed effects logistic regression model for effects of farming and food preparation and safety practices and occupation on seropositivity to brucellosis in Azerbaijan with location entered as a random effect.

Predictor variables	Level (number)	Odds Ratio (95% CI)	*P* value
Keep small ruminants	Yes (1595)	3.5 (1.6, 7.9)	<0.002
	No (767)	Reference	
Make raw milk cheese	Yes (143)	3.9 (1.7, 8.9)	0.001
	No (2219)	Reference	
Use fresh cheese	Yes (294)	86.4 (40.1, 186.1)	<0.001
	No (2068)	Reference	
Heard educational information	Yes (702)	0.1 (0.0, 0.2)	<0.001
	No (1660)	Reference	
Slaughter animals	Yes (192)	15.3 (6.2, 37.3)	<0.001
	No (2170)	Reference	
Vaccinate against brucellosis	Yes (349	0.01 (0.01, 0.02)	<0.001
	No (2013)	Reference	

associated with infection in winter pasture flocks and high rates of use of raw milk and sale of milk products made with raw milk and provided information for incorporation into public awareness programs which are part of the national brucellosis control program. It involved farmers, private and Ministry of Agriculture veterinarians and a post-graduate student from the Azerbaijan State Agrarian University and demonstrated the value of epidemiology research for solving country-specific problems and making evidence-based decisions. Risky practices for humans were demonstrated by high prevalences of use of raw milk, consumption of fresh cheese, sale of cheese in village and local markets. Use of raw milk is a risky practice, not only for brucellosis, but also for campylobacteriosis, listeriosis, salmonellosis and *E. coli* infection (Dadar et al., 2019; van den Brom et al., 2020; Verraes et al., 2015). Infection in some of the control farms cannot be ruled out given the sample size of 100 mature female small ruminants but the effect of misclassification would be bias towards the null. An encouraging finding for authorities was expression of willingness to contribute to the costs of elimination of brucellosis by 178 of the 196 farmers in the case control study.

We consider the 8.1% seroprevalence estimate for humans to be a reasonably accurate representation of the seroprevalence in all regional locations in Azerbaijan, given the number of persons tested (11,270) and the use of random sampling, albeit with some bias likely for doctors and nurses, throughout all stages for selection of participants. The high number of human health worker participants (584 nurses and 206 doctors) suggests that they may have been over-represented in the survey population. Nurses and doctors were involved in taking samples and it is likely that they took the opportunity to check their own status.

Sample sizes of 60 for regional towns and 45 for villages were designed to give broad coverage of regional towns and villages and produce a reliable estimate for the whole country. Individual location estimates of human prevalences in villages and regional towns have wide confidence intervals but the range of numbers of seropositives from a median of 3 to 18 within villages reflects the wide variation and clustering of infection observed among village-based livestock and winter pasture flocks in all livestock serosurveys. The total number of seropositives for the four regional cities was 25, giving an overall estimate of 5.7% (3.9, 8.3). A lower prevalence was expected in regional cities than in regional towns and villages because of probable lesser contact with animals but a larger sample size would be required to produce a more precise estimate. The regional city prevalence of 5.7% is plausible and in line with expectations, but it is still high and therefore of concern to public health and veterinary authorities. The similar prevalences in persons from villages and regional towns probably reflects the close relationships among persons in those locations.

The higher prevalence in males than in females recorded has been reported elsewhere (Dastjerdi et al., 2012) and for Azerbaijan is thought to be due to more opportunities for exposure in males to sources of infection from animal products and during husbandry of animals. The relatively even level of prevalences for all age groups, with the exception of the 10:14 year-olds, is interesting because seroprevalences could be expected to increase with increasing age due to more opportunities for exposure to infection with increasing age. It is not known for how long Brucella titres persist in humans and it may be that the even prevalence level recorded here is due to titres declining over time and limited opportunities for additional exposures to infection. The 8.8% prevalence for persons aged between 10 and 19 years indicates exposure to infection at a relatively early age. The wide confidence intervals around estimates of 2.3% (0.5, 6.7) for the 10:14 age group and 11.8% (8.2, 15.3) for the 15:19 group are due to small samples sizes, but the data indicate exposure at relatively early ages. Follow-up studies of onset of infection in young persons could provide useful insights into the epidemiology of the disease in humans and sources of risk of exposure to infection. The multivariable analyses indicated that risks were higher for persons in farm related occupations and for males, and lower for village residents than for regional centre town and city residents.

A brucella related antibody titre detected by the RBT and the enzyme immunoassays provides evidence of exposure to brucellae but not to the time of exposure to infection and a cautious approach is warranted when interpreting associations between positive test and occupations. A person in an office occupation at the time of testing may have had previous contact with animals in the past, e.g. looking after animals on a farm. Nevertheless, the associations that the study has shown between positive tests, gender, and occupations involving close contact with animals and/or preparation of animal products are biologically plausible and in line with similar studies in other countries (Abedi et al., 2020; Al-Shamahy et al., 2000; Husseini and Ramlawi, 2004; John et al., 2010). The high-risk occupations identified in this study warrant increased surveillance and persons from those occupations should be routinely tested for brucellosis if they experience fever and symptoms suggestive of brucellosis. A collaborative One Health response involving public health and veterinary authorities is warranted when disease occurs in either animals or humans (Godfroid et al., 2013; WHO et al., 2019).

Analysis of the smaller subset of persons who had farms showed that persons with farms and keeping small ruminants increased the risk of positivity but keeping only cattle was not associated with risk. Associations between seropositivity and the effects of farming, food preparation and occupation were analysed in the data subset of 2362 persons and risks were associated with keeping small ruminants, slaughtering of animals, making cheese from unpasteurised milk and using fresh cheese, whereas having heard educational information and vaccinating against brucellosis were protective. The greatest risks for testing positive were using fresh cheese and involvement with slaughter of animals.

Although there was a general strong awareness of brucellosis, knowledge about symptoms of brucellosis in animals was generally poor. However, there was a better understanding of the factors associated with humans becoming infected among. There was a strong perception that uncooked meat is risky, but unlike unpasteurised milk and milk products, consumption of uncooked meat has not been shown to be associated with transmission of brucellosis. Important findings from the study about risk of infection the subset of 4800 persons who had heard about brucellosis from doctors, TV, veterinarians, newspapers and school were production of cheese from unpasteurised milk by 6.1% of 2362 persons, use of unpasteurised milk by 18% of 11,270 participants and consumption of fresh cheese by 11.4% of 11,270 participants. Contact with animals at birthing and slaughtering and consumption of unpasteurised milk and milk products are known to be the main methods of transmission of infection to humans (Abedi et al., 2020; Al-Shamahy et al., 2000; Husseini and Ramlawi, 2004; John et al., 2010), and although the study did not investigate the amounts of unpasteurised milk and milk products offered for sale, it is clear that more official regulation will be required to help to reduce risks associated with these products.

The study improved the understanding of brucellosis in humans in Azerbaijan and was helpful for public health and veterinary authorities. Although not all infections with brucellosis result in serious disease, the high prevalence of seropositive persons and the early age of exposure clearly identifies the disease as a significant public health and veterinary problem. Brucellosis can be controlled and reduced to very low levels in animals and implementation of targeted food safety regulations can reduce the risk associated with sales of risky milk and milk products.

Literature searches were used wherever possible to guide decision making for the control programme and uncertainties and conflicting advice from various consultants and international agencies were resolved after careful consideration of likely benefits and costs. Contentious issues included vaccination of male sheep, repeat vaccination of cattle with conjunctival Strain 19 and use of RB51. Absences of peer-reviewed published evidence to support transmission from male small ruminants to females, enhanced protection from repeat vaccination of cattle or any advantage for RB51 over Strain 19 were taken into account along with benefit cost analyses to reject these recommendations.

The Azerbaijani have achieved steady progress with control of brucellosis control despite a complex array of social, political and fiscal constraints that are not always appreciated by outside consultants. Safe handling of livestock, especially around birthing periods, and pasteurisation of milk products have been promoted through public awareness programmes and an important and very encouraging finding from the studies has been livestock owner support for elimination of disease in their animals and willingness to share costs with government. The most recent initiative is privatisation of most of the SVCS field veterinarians with contracts for services such as vaccination. Consideration is now being given for the use of predictive benefit cost models for assessment of options for control that include voluntary accreditation as a preparatory stage prior to moving to a mandated test and slaughter control programme with partial cost recovery from livestock owners. An essential component of the mandated programme will be quality assurance of all laboratory test procedures in veterinary and public health laboratories.

Elimination programmes that were successful in well-resourced countries are inappropriate for countries such as Azerbaijan where a different model that takes the complex web of constraints into account is required. The Azerbaijan experience has shown that patient progress can be made and hopefully look forward for further reductions in their wish list for improvements to the programme over the next few years. The Azerbaijanis have been open in reporting their experiences in this paper and trust that it will help other countries to deal with constraints and promote confident expectations for their control programmes.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rvsc.2021.05.014.

References

Abedi, A., et al., 2020. The prevalence of Brucella spp. in dairy products in the Middle East region: a systematic review and meta-analysis. Acta Trop. 202.
Abernethy, D.A., et al., 2012. Field trial of six serological tests for bovine brucellosis. Vet. J. 191 (3), 364–370.
Al-Shamahy, H.A., Whitty, C.J.M., Wright, S.G., 2000. Risk factors for human brucellosis in Yemen: a case control study. Epidemiol. Infect. 125 (2), 309–313.
van den Brom, R., et al., 2020. Zoonotic risks of pathogens from sheep and their milk borne transmission. Small Rumin. Res. 189.
Cooper, C.W., 1992. Risk factors in transmission of brucellosis from animals to humans in Saudi Arabia. Trans. R. Soc. Trop. Med. Hyg. 86 (2), 206–209.
Dadar, M., Shahali, Y., Whatmore, A.M., 2019. Human brucellosis caused by raw dairy products: a review on the occurrence, major risk factors and prevention. Int. J. Food Microbiol. 292, 39–47.
Dastjerdi, M.Z., Nobari, R.F., Ramazanpour, J., 2012. Epidemiological features of human brucellosis in Central Iran, 2006-2011. Public Health 126 (12), 1058–1062.
Godfroid, J., et al., 2013. A "one health" surveillance and control of brucellosis in developing countries: moving away from improvisation. Compar. Immunol. Microbiol. Infect. Dis. 36 (3), 241–248.
Hernandez-Mora, Gabriela, et al., 2017. Epidemiology of bovine brucellosis in Costa Rica: lessons learned from failures in the control of the disease. PLoS One 12 (8).
Husseini, A.S., Ramlawi, A.M., 2004. Brucellosis in the West Bank, Palestine. Saudi Medical Journal 25 (11), 1640–1643.
Jimenez de Bagues, M.P., et al., 1989. Responses of ewes to B. melitensis rev 1 vaccine administered by subcutaneous or conjunctival routes at different stages of pregnancy. Annales de Recherches Veterinaires 20 (2), 205–213.
John, Kunda, et al., 2010. Quantifying risk factors for human brucellosis in rural northern Tanzania. PLoS One 5 (3).
Manual, Terrestrial, 2018. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Aceessible at. https://www.oie.int/international-standard-setting/terrestrial-manual.
Nejad, R.B., et al., 2020. Brucellosis in the Middle East: current situation and a pathway forward. PLoS Negl. Trop. Dis. 14 (5).
Nielsen, K., 2002. Diagnosis of brucellosis by serology. Vet. Microbiol. 90 (1–4), 447–459.
OIE, 2008. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Chapter 2. 4. 3. page 636). http://www.oie.int/eng/normes/mmanual/A_summry.htm (accessed 30th November 2008).
Pappas, G., et al., 2006. The new global map of human brucellosis. Lancet Infect. Dis. 6 (2), 91–99.
Racloz, V., et al., 2013. Persistence of brucellosis in pastoral systems. Rev. Scient. Tech. (Int. Off. Epizoo.) 32 (1), 61–70.
van Straten, M., et al., 2016. Brucella abortus S19 vaccine protects dairy cattle against natural infection with Brucella melitensis. Vaccine 34 (48), 5837–5839.
Verraes, C., et al., 2015. A review of the microbiological hazards of dairy products made from raw milk. Int. Dairy J. 50, 32–44.
WHO, FAO, OIE, 2019. Taking a Multisectoral, One Health Approach: A Tripartite Guide to Addressing Zoonotic Diseases in Countries. Acessible at. https://www.who.int/publications/i/item/taking-a-multisectoral-one-health-approach-a-tripartite-guide-to-addressing-zoonotic-diseases-in-countries.