

Seroprevalence of Brucellosis in Small Ruminants Across Three Agro-Climatic Zones

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ABSTRACT

*Brucellosis caused by *Brucella melitensis* is one of the commonest infectious causes of abortion in sheep and goats, and the same animals are a main source of human infection through raw milk and contact with birth fluids. Surveys of antibodies in small ruminants are therefore widely used to describe the problem, and many of them are read as showing that infection is commoner in one kind of farming country than another. This narrative review asks how far that reading can be trusted when flocks from arid rangeland, mixed-farming plains and wetter hill country are compared. It draws on published work on the biology and transmission of the organism, on serological testing, on the design and analysis of prevalence surveys, and on the livestock systems typical of each zone, and it reports no new data. Differences between zones are biologically plausible, since mixing at water points, housing density, trade and the presence of cattle all change exposure. They are also entangled with the choice of test, the sampling frame, the unit of analysis, the vaccination history and the year. The review concludes that a zone effect is credible but rarely demonstrable from existing reports, and it sets out what a survey needs to record before its figures can be compared with those of another.*

Keywords: Brucellosis; Small ruminants; Seroprevalence; Agro-climatic zones; Serological tests; Survey design; Narrative review

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1. Introduction

Brucellosis is an old disease that keeps finding new places to live. The genus *Brucella* has been known since the nineteenth century as the cause of Malta fever, and its list of hosts has only grown since, to include marine mammals as well as farm animals [1]. In sheep and goats the usual culprit is *Brucella melitensis*, an organism that causes abortion, stillbirth and weak young, and that passes readily from animals to people [2, 3]. Human illness is real and often prolonged, with fever, joint pain and fatigue that can be mistaken

for many other conditions [4]. Reviews of the global picture agree that the burden falls hardest where people live close to their animals and drink their milk unboiled [5, 6].

Because infected animals are often silent carriers, the usual way to find out how widespread the infection is, is to draw blood and look for antibodies. A survey of this kind returns a proportion, the seroprevalence, and that number is quoted in reports, in grant applications and in control plans. Quoting is easy. Comparing is harder, and the comparison that interests many

veterinary authorities is geographic: is brucellosis in small ruminants commoner in dry rangeland, in the mixed-farming plains or in the wetter hills? The answer would help to decide where scarce vaccine and staff should go [7, 8].

This review looks at that question with some suspicion. A difference between zones is biologically plausible, since climate and farming system shape how animals meet each other and how long the organism survives outside the host [9]. But a difference between published figures is not automatically a difference between zones. The figures come from different tests, different sampling schemes and different years, and zones are rarely the only thing that changes when a team crosses a district boundary. We ask how much of a zone effect survives once those other sources of variation are acknowledged.

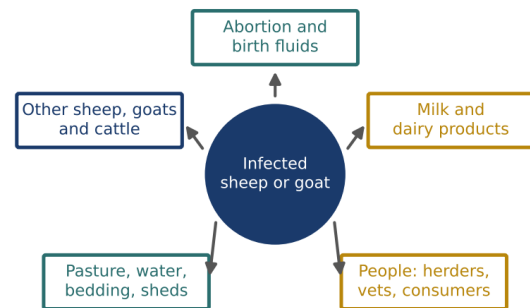
The article is a narrative review. It draws on published work on the organism, the host, serological testing and the design of prevalence surveys, and it deliberately reports no new data and no prevalence figures of our own. Where the literature is thin or conflicting, we say so, and we describe the information that a future survey should record so that its results can be placed beside others.

2. Literature Review

2.1 The Organism, the Host and the Routes of Spread

Brucella melitensis is the principal agent of brucellosis in sheep and goats, although *B. abortus* can infect them when they share pasture with infected cattle, and *B. ovis* causes a distinct genital infection of rams that is not a human hazard [10, 11]. The bacterium is a facultative intracellular parasite. It survives inside the host's macrophages and in cells of the placenta, which helps to explain both its persistence in the animal and its concentration in birth tissues [12, 13].

The main source of infection is the abortion or birth itself. Fetal membranes, fluids and the fetus carry very large numbers of organisms, and these contaminate bedding, pasture and water, where other animals sniff, lick or ingest them [13]. Milk is another route, both to suckling young and to people, which is why unboiled milk and fresh cheese appear so often in accounts of human cases [14]. Figure 1 sets out these routes in outline. Venereal spread seems to matter less in small ruminants than the other routes, and transmission through the air in crowded sheds is probably more important than is usually allowed [1].



Schematic of routes, not data

Figure 1. Routes by which *Brucella* moves between small ruminants, cattle, the environment and people (schematic, not data).

Two features make surveys difficult. First, many infected animals never abort, or abort only once, so that clinical history underestimates exposure. Second, the antibody response follows the infection by some weeks, so that an animal sampled early may be negative and one sampled long after an abortion may remain positive [15, 16]. A cross-sectional figure is therefore a mixture of recent, old and resolved infections, and the mixture changes with the season in which the flock was visited.

2.2 Livestock Systems in Contrasting Zones

Small ruminants are kept in very different ways across the world's climates. Maps of livestock distribution show sheep and goats spread across dry, humid and mountainous regions alike, and the animals matter most to the households with the fewest alternatives [17, 18]. For the purposes of this review we use three generic zone types, summarised in Table 1: arid and semi-arid rangeland, mixed-farming plain, and humid hill country. These are not official classifications. Official agro-climatic zones differ from country to country, and climate-only schemes such as the Köppen-Geiger maps use other criteria again [19, 20]. Our types are a device for thinking about exposure.

In rangeland, flocks move. They meet at wells and markets, they share grazing, and a single infected female can seed an outbreak across several herds. Work on pastoral systems has argued that this mixing, combined with limited veterinary reach, helps infection persist even when each owner has few animals [21, 22]. In the plains, flocks are smaller and often stall-fed, but they share common land and sit beside cattle and buffaloes, and village animal numbers can be high [23, 24]. In hill country, holdings are scattered and mixing may be lower, though animals are often sheltered in the cold season and the shared air of a small shed may do the work that mixing does elsewhere [9].

Table 1. Three illustrative zone types and the features that could shape exposure to *Brucella*.

Feature	Arid and semi-arid rangeland	Mixed-farming plain	Humid hill country
System	Mobile or semi-mobile flocks; communal grazing [21, 22]	Small flocks near villages; stall feeding and grazing [23, 24]	Small herds on slopes; seasonal movement
Mixing of animals	High at water points and markets [21]	Moderate; shared common land	Low to moderate; scattered holdings
Housing	Open yards; crowding at birth	Sheds at night; crowding at kidding [9]	Often sheltered in cold or wet seasons
Cattle nearby	Variable	Often present	Often present
Vet services	Often limited [37]	Better, but uneven	Often limited by terrain

Note: The zone types are generic categories chosen for this review, not official agro-climatic classifications; features are qualitative.

2.3 Serological Testing in Sheep and Goats

The Rose Bengal test is the usual first step because it is cheap, quick and can be done in a village. It detects antibody to the smooth surface lipopolysaccharide of the bacterium, and it is regarded as a screening method whose positives should be confirmed [15, 25]. Complement fixation and enzyme-linked immunosorbent assays are used for confirmation, and their strengths and weaknesses are summarised in Table 2 and Figure 2. None of the tests is perfect, and the literature on ruminant serology stresses that the same serum can give different answers on different platforms [16].

Table 2. Serological tests commonly used in small-ruminant surveys and what each can and cannot do.

Test	Main strength	Main limitation
Rose Bengal	Cheap, fast, usable in the field [25]	Screening only; false positives possible [15]
Complement fixation	Long-standing reference method [15]	Needs a laboratory and trained staff
Indirect ELISA	Sensitive; suits large batches [16]	Cross-reaction with other smooth bacteria
Competitive ELISA	Better discrimination in some settings [16]	Kit and cut-off choices matter
Fluorescence polarisation	Rapid, single-step	Equipment cost; less used in small flocks

Note: Qualitative summary from the cited diagnostic literature; performance depends on antigen, cut-off and population.

Two sources of error deserve emphasis. Smooth bacteria of other genera, such as *Yersinia enterocolitica* O:9, share surface antigens with *Brucella* and can produce false positives [15]. And vaccination with the live Rev 1 strain, which is widely used in sheep and goats, produces antibodies that the tests cannot tell from those of infection unless the dose, route and age at vaccination are known [26, 27]. A survey in a zone with an active vaccination campaign is not measuring the same thing as one in a zone without.

2.4 What Prevalence Surveys Can and Cannot Say

A seroprevalence is an apparent proportion. It becomes an estimate of the true proportion of infected animals only after correction for the sensitivity and specificity of the test, and the correction is possible only when those properties are known for the population studied [28, 29]. When no gold standard exists, as is the case here, latent-class methods can estimate test properties and prevalence together, but they rest on assumptions that need to be stated [30].

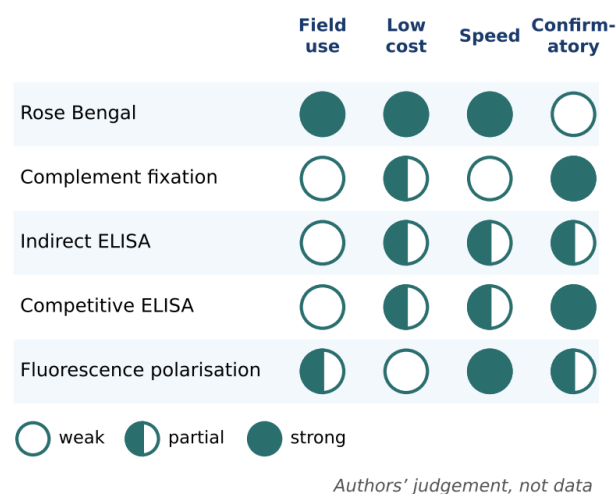


Figure 2. Qualitative grid of serological tests against practical criteria (authors' judgement, not data).

Precision is a second matter. Small ruminants live in flocks, and animals in the same flock resemble each other more than they resemble animals elsewhere. A survey that treats each sampled animal as an independent observation will report intervals that are too narrow, which is a version of the pseudoreplication problem described long ago in ecology [31, 32]. Even a correct design needs an interval that behaves well at the small and large proportions typical of disease surveys, and the common textbook interval does not [33].

2.5 Regional Evidence and Its Patchiness

Regional reviews give a sense of both the burden and the unevenness of the evidence. Reviews for the Middle East and for Kenya describe wide variation between studies, and they attribute part of it to differences in design and in the animals sampled as well as to real differences in risk [34, 35, 36]. Sub-Saharan reviews note the shortage of reliable diagnostics and of control programmes [22, 37]. For India, reviews point to the large number of small ruminants, the estimated economic losses to livestock keepers, and the scarcity of consistent national data [23, 24]. Wider commentary on the geography of the disease emphasises that it is emerging or re-emerging in several regions at once, in step with a wider rise in zoonotic disease [5, 38, 39].

3. Materials and Methods

3.1 Scope and Question

The review asks one question in two parts. Do published surveys of small ruminants support the idea that the seroprevalence of brucellosis differs between arid rangeland, mixed-farming plain and humid hill country? And, if they do, how much of the apparent difference could be produced by the way the surveys were done? The second part is the more useful one for anyone planning a survey, because it identifies what must be recorded.

3.2 Literature Assembly

We drew on peer-reviewed work in four areas: the biology and transmission of *Brucella* in small ruminants; serological testing; the design and analysis of prevalence surveys; and regional reviews of brucellosis in livestock and people. We did not run a formal systematic search and we do not claim one. This is a narrative synthesis, and the choice of sources reflects the authors' judgement, so the review is a better guide to the structure of the problem than to the size of any effect.

3.3 Appraisal Framework

To judge how far a survey figure could be compared with another, we asked four questions. How were flocks and animals chosen, and was the reason for sampling stated? Which test was used, with what cut-off, and had it been validated in this population [29, 30]? Was vaccination status known [27]? And was clustering within flocks recognised in the analysis [31, 32]? The answers shaped the reading presented in Table 3 and Figure 3.

4. Results

4.1 Is There a Plausible Zone Effect?

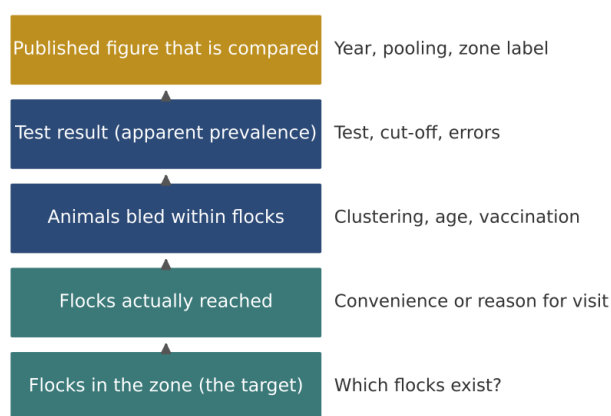
Yes, on biological grounds. Exposure is driven by contact with infected birth material and milk, and each zone type shapes contact differently. Mobility and communal use of water favour the spread of infection between herds [21]. Crowding at birth in sheds favours it within a herd [9]. The presence of cattle can introduce *B. abortus* into mixed holdings [10]. Climate also acts on the organism outside the host, since heat and sunlight reduce survival while cool, damp and shaded conditions favour it, though the size of that effect on the risk to animals is poorly documented [1].

A zone effect is therefore not an arbitrary idea. What the literature does not provide is a set of surveys designed to isolate it. Studies were generally planned to describe one district, or to compare species, or to support a control decision. They were not planned to compare zones, and when a review lines them up by zone it inherits all the differences that came with them. The inheritance is silent, which is exactly why it is so easily overlooked.

4.2 How the Evidence Is Entangled

Zone is entangled with production system. Dry rangeland tends to mean mobile flocks, while mixed farming tends to mean small sedentary ones, so any difference could be due to either [21, 23]. It is entangled with the cattle population, which brings in a second species of *Brucella* [10]. It is entangled with access to veterinary services, which governs both the vaccination history and the quality of laboratory work [37]. And it is entangled with the reason for sampling: surveys prompted by abortions in a district find more than surveys of apparently healthy flocks, and the former are more likely in places with reporting systems. A veterinarian reading a survey report needs the answer to a simple question: would the same figure turn up in my district?

The testing issue compounds the problem. Two surveys that report a similar proportion with a screening test and a confirmatory test are not reporting the same quantity, and a pooled comparison of such reports may amount to a comparison of laboratories [15, 16]. If one zone is sampled mainly with a screening test, which tends to give some false positives, an apparent excess there may be partly an artefact of the method [25]. That is a modest point, yet it is enough to blur any ranking of zones.



Schematic: each layer can distort the one above; not data

Figure 3. Layers between a flock and a published prevalence figure; each adds a possible distortion (schematic, not data).

Table 3. Where bias enters a survey and how a report can make it visible.

Source	Effect on the figure	What to report
Convenience sampling	Over- or under-states prevalence	How flocks and animals were chosen
Sampling after abortions	Inflates apparent prevalence	Reason each flock was visited
Animals as independent units	Overstated precision [31, 32]	Flock-level clustering; intervals [33]
Imperfect test	Apparent differs from true prevalence [28, 29]	Test, cut-off, validation [30]
Vaccination history	Antibodies without infection [26, 27]	Vaccine, route, age at dosing
Pooling of years	Hides trends and outbreaks	Year of sampling

Note: Sources are those discussed in the cited methodological literature; they are not attributed to any single survey.

4.3 Sampling, Clustering and Precision

Many survey reports in the regional reviews rely on convenience sampling, such as flocks that were easy to reach or animals brought to a market or a clinic [34, 35], so that the figure describes the sample more than the zone. Table 3 lists the main sources of distortion and what a report can do about each.

Clustering gets less attention than it deserves. If the flocks differ in their infection status, which they usually do because infection often concentrates in a few flocks, then the effective sample size is closer to the number of flocks than the number of animals [32]. A survey of many animals in few flocks is thus much less informative than it looks, and the interval attached to its prevalence should reflect that [31, 33]. The

correction is unglamorous, but it changes how much weight a figure can bear.

4.4 Vaccination and Time

Where Rev 1 has been used, serology cannot separate vaccine from field infection unless the vaccination record is clear [26, 27]. Zones with active programmes will therefore show antibodies in animals that are not infected, while zones without them will show only the antibodies of infection. The direction of the resulting bias depends on how long ago vaccination took place and on the age of the animals sampled, details that reports seldom give.

Time is the other neglected variable. The expected pattern after a good control programme is a fall in prevalence over several years, while an outbreak will produce a spike that fades [40]. A survey is a snapshot, and two snapshots from different years can differ for reasons unrelated to climate or farming. Pooling years, which regional reviews are often forced to do, hides trends of exactly this kind [34, 35]. The point is not that a survey is poor, only that its figure travels less far than its abstract suggests.

4.5 A Cautious Reading

Put plainly, the literature supports a cautious statement. Brucellosis in small ruminants is present in every kind of farming country covered by the reviews we cite [5, 6, 38]. There are good biological reasons to expect that exposure differs between production systems, and these systems partly follow climate. The surveys themselves are rarely good enough to say by how much, and the apparent differences between zones are, in our reading, better treated as hypotheses than as findings. This is why the same disease can look common in one report and unremarkable in the next.

5. Discussion

5.1 Interpretation and Practical Reading

The main finding of this review is a conditional one. A zone effect is credible, and it probably runs through production system rather than climate in any direct sense. A field veterinarian who knows that mixing at water points, crowding at birth and contact with cattle drive exposure can act on these things whatever the labels on the map say [7, 21]. For those drawing national maps, the more honest reading is that existing figures show where infection has been found, not where it is most common.

The second point concerns control. Vaccination decisions are usually made on prevalence, and the

prevalence is usually apparent prevalence from a screening test. Where the test is poor or the sampling biased, the choice between test-and-slaughter and mass vaccination may be made on a figure that is wrong in one direction or the other [40, 41]. The cost of a wrong decision is borne by herders and, through milk and cheese, by their customers [14, 42]. A prevalence that cannot be reproduced with a second test is not one a control programme should be built on.

Three findings would change this reading. A set of surveys using one test and one design across zones, with flock as the unit, would test the zone effect directly. A clear demonstration that climate acts independently of production system would shift attention from management to environment. And a good validation study of the usual screening tests in local sheep and goats would reduce the uncertainty that now surrounds every figure. Reviews of this kind are more useful when they say where the evidence stops than when they smooth it over.

5.2 Limitations

This is a narrative review and shares the limitations of that form. The selection of sources reflects judgement, no formal search or quality scoring was done, and the review does not quote numerical results from individual surveys, because those could not be checked against the originals within this work. Our three zone types are a thinking device and not a classification, and a reader who works with an official classification will find that boundaries do not coincide. Where the evidence rests on reviews and on cattle or human data, the mechanisms proposed here should be read as plausible rather than established. Read this way, a low figure in a small survey is not evidence that the disease is absent.

5.3 Implications for Survey Design

A survey meant to compare zones would sample flocks, not just animals, by a stated random or systematic scheme within each zone, and would record the number of flocks as the main design quantity [31, 32]. It would use a single test protocol, ideally with a screening and a confirmatory step, and report the cut-off and any validation [29, 30]. It would record vaccination history flock by flock, so that animals with vaccine antibodies could be identified or excluded [27]. Apparent and corrected prevalence would both be reported, with an interval that respects clustering [28, 33], and the year, season and reason for each visit would be stated. It is a habit worth keeping even when the answer seems obvious.

5.4 Directions for Future Work

Three needs stand out. First, a formal systematic review with a transparent search and appraisal of small-ruminant surveys, ideally able to model between-study differences instead of ignoring them. Second, coordinated surveys in several zones with a common protocol, designed from the outset to compare them, and tied to existing One Health surveillance where possible [7]. Third, validation studies of serological tests in local breeds and under local vaccination practice, so that corrected prevalence can be reported with confidence [16]. A flock that has been sampled once says little about the same flock a year later. Nothing in this reading argues against surveying; it argues for describing what was done.

6. Conclusion

A zone effect on the seroprevalence of brucellosis in small ruminants is plausible, because mixing, housing, trade and the presence of cattle all shape exposure and all vary with farming system. The evidence, as we can responsibly summarise it, does not yet establish the size of that effect. Differences between published figures are as likely to reflect tests, sampling and vaccination as zones, and they should be read as hypotheses.

For the practitioner, the message is practical rather than discouraging: record how animals were chosen, which test was used, what the flock's vaccination history is and when sampling took place. For the researcher, the opportunity is a set of surveys built to compare zones, using flocks as the unit and reporting uncertainty honestly.

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9. Conflict of Interest

The authors declare no conflict of interest.

10. Author Contributions

G.S. conceived the review and wrote the sections on transmission and serological testing. A.G. wrote the sections on laboratory methods and vaccination. K.K. wrote the sections on reproductive disease, survey design and field practice. All authors revised the manuscript and approved the final version.

11. Ethics Statement

This is a review of published literature. No animals were used and no ethical approval was required.

12. Data Availability

No new data were generated. All sources are cited in the reference list.

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