Veterinary Record Open

Methodological aspects of serosurveillance in resource-poor settings

Susanna Sternberg Lewerin,[1] Cecilia Wolff,[1] Charles Masembe,[2] Karl Ståhl,[3] Sofia Boqvist,[1] Mikael Andersson Franko[4]

To cite: Sternberg Lewerin S, Wolff C, Masembe C, *et al*. Methodological aspects of serosurveillance in resource-poor settings. *Veterinary Record Open* 2018;**5**:e000273. doi:10.1136/vetreco-2017-000273

Received 28 December 2017
Revised 13 March 2018
Accepted 20 March 2018

[1]Department of Biomedical Sciences and Veterinary Public Health, Swedish University of Agricultural Sciences, Uppsala, Sweden
[2]College of Natural Sciences, Makerere University, Kampala, Uganda
[3]Department of Epidemiology and Disease Control, National Veterinary Institute SVA, Uppsala, Sweden
[4]Department of Applied Statistics and Mathematics, Swedish University of Agricultural Sciences, Uppsala, Sweden

Correspondence to
Professor Susanna Sternberg Lewerin; susanna.sternberg-lewerin@slu.se

ABSTRACT

Animal production is important for the agricultural economy in low-income countries, but is threatened by infectious diseases. Serosurveys are conducted for different reasons such as disease detection, risk factor studies, disease monitoring and establishing disease-free status. Most reports on such serosurveys include some discussion about methodological constraints but still, by necessity, rely on serological results for case definition. This study uses a cross-sectional serosurvey for foot-and-mouth disease (FMD), Rift Valley fever (RVF) and contagious bovine pleuropneumonia (CBPP) in cattle in three districts in Western Uganda to illustrate the limitations of this approach, addressing the questions of what flaws can be expected in sampling and diagnostics and how these influence the results. The target was to collect blood samples from 60 cattle herds per district. To reflect the recent infection history of the herd, young animals (two to five years) were prioritised. The farmers were interviewed about management, cattle trade, cattle health and vaccination. Commercial ELISAs were used for serological analyses: for CBPP the IDEXX CBPP *Mycoplasma mycoides* subspecies mycoides antibody test kit, for RVF the ID Screen Rift Valley Fever competitive ELISA, and for FMD the PrioCHECK FMDV NS. Apparent prevalence, true prevalence and associations with herd characteristics were assessed. The sampling plans could not be entirely fulfilled, nor the number of tests run in the laboratory. There were reactors to all three diseases with an apparent prevalence of approximately 30 per cent for CBPP, 6 per cent for RVF and 7 per cent for FMD. Calculation of true prevalence based on test sensitivity and specificity resulted in a slightly higher prevalence figure for CBPP and lower figures for RVF and FMD. The study illustrates the importance of considering diagnostic test performance when interpreting results from serosurveys, and the challenge of representative sampling and laboratory work in low-income countries.

INTRODUCTION

In low-income countries, infectious diseases threaten livestock production via direct effects for the farmer as well as other actors in the complex value chains of animal products.[1] Outbreaks of transboundary animal diseases may have additional consequences for the farmers due to trade restrictions and, in addition to clinical surveillance, serosurveillance is often conducted to establish disease status.[2] The methodological challenges in association with sampling design, collection and transport of samples and diagnostic test characteristics are exacerbated in poor-resource settings. Although this is well known to most researchers working in such settings, some aspects may not be obvious to others and published data may be misinterpreted or not sufficiently questioned or validated. Hence, there is a gap between advanced epidemiological research and basic descriptive studies in resource-poor settings.

The bovine disease spectrum in Uganda is similar to many other sub-Saharan African countries and dominated by infectious diseases. Foot-and-mouth disease (FMD) is controlled by vaccination and quarantine, but inadequate vaccination coverage leads to repeated outbreaks.[3 4] Moreover, there are indications of the presence of FMD in animal species (goats, sheep) that are not routinely included in vaccination campaigns.[3] Contagious bovine pleuropneumonia (CBPP) is present in Uganda, although apparently under-reported.[5 6] The disease is controlled by vaccination and movement restrictions, but vaccination was not in place at the time of the study.[7] Rift Valley fever (RVF), which is mainly vectorborne, is endemic in the neighbouring countries Kenya and Tanzania and some parts of Uganda have been defined as suitable for RVF spread.[8] Vaccination is used to control RVF in endemic regions but was not in place in the study area at the time of the study.[7] Serological reactions to RVF virus (RVFV) were identified in goats in areas close to the study region in 2009–2011[9] and in 2016, after this study was conducted, RVF was reported south of the study region, first in humans and later in animals.[6 10] In November 2017, RVF was confirmed in two people in the districts of Mityana and Kiboga, east of the study area.[11]

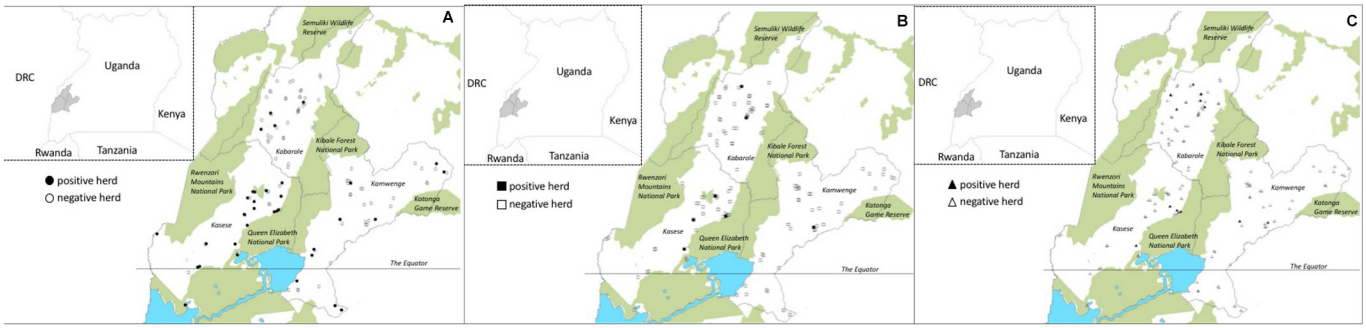

FIG 1: Maps of study area showing the location of positive and negative cattle herds (a, contagious bovine pleuropneumonia [CBPP]; b, Rift Valley fever [RVF]; c, foot-and-mouth disease [FMD]) in a serosurvey conducted in 2015.

While CBPP, RVF and FMD differ in transmission routes, host range and probability of transmission between herds, they are all highly relevant for cattle production and recognised by most farmers, not least due to the usually obvious clinical signs. As the diseases are subject to official control and/or governed by trade guidelines, serosurveillance is relevant for monitoring.

The aim of this study was to illustrate the caveats of using serological surveys for assessment of prevalence and risk factors for animal diseases in resource-poor settings using a serosurvey for CBPP, RVF and FMD in three districts in Western Uganda as an example. The research questions were: what flaws can be expected in sampling and diagnostics and how do these influence the results?

MATERIALS AND METHODS

Sampling

In 2015, we investigated the seroprevalence of CBPP, RVF and FMD in parallel with a study on biosecurity practices among cattle farmers in Western Uganda.[12] The selection of herds was made as previously described[12] and summarised below. The study area included the districts Kabarole, Kamwenge and Kasese in South-Western Uganda, an important area for livestock production (Fig 1a–c). Each district is organised in the administrative units of subcounties divided in parishes that are further divided in villages. A 'village' is an administrative term that is not reflected in any obvious clustering of people or animals.

The study used a cross-sectional design. For sample size estimations, district cattle population sizes from the livestock census[13] and herd size estimations from each district veterinary officer (DVO) were used (see Table 1). Prior assumptions about true prevalence were based on literature data.[5 9 14] For calculation of the sample size to estimate herd prevalence, the online tool Epitools (epitools.ausvet.com.au) was used. Confidence levels of 95 per cent and 1–10 per cent precision, herd sensitivity (HSe) and herd specificity (HSp) in the range of 0.7–0.999 and true prevalences from 0.1 to 30 per cent were evaluated. A total sample size of 180 herds, that is, 60 per district, was decided upon, based on what would be practically feasible and allow a reasonably precise prevalence estimation. Epitools was also used to calculate the number of animals to be tested in a herd to detect at least one positive animal. Herd sizes between 10 and 100, based on previous information about the herds in the three districts, were evaluated with a confidence level of 95 per cent, desired HSe of 95 per cent and test sensitivity of 98 per cent. The final sample size was: all animals in herds with up to 20 cattle; 20 animals in herds with 21–50 cattle; and 30 animals in herds with more than 50 cattle.

A two-stage selection process was used, with a simple random selection of 30 villages in each district followed by a random selection of two herds per village using the random number function in Microsoft Excel (Microsoft, Redmond, USA). In Kasese district, the DVO reported that 75 per cent of the initially selected villages had no cattle. Instead, a purposive sample of the remaining villages which the DVO confirmed had at least two cattle herds was made, geographically spread over the district. Each selected village was visited by a local veterinary officer or veterinary assistant. A sampling frame was created by asking the village chairperson to list all farmers owning at least two cattle. Herds were

TABLE 1: Number of herds and herd sizes as provided in official statistics,[13] estimated by DVOs in 2015 and originally sampled in 2015[12]

	Kabarole	Kamwenge	Kasese
Official statistics			
Number of herds	15,530	14,100	5530
Mean (median) herd size	4.3 (3)	8.6 (4)	17.6 (11)
DVO estimates			
Number of cattle	60,000	120,000	100,000
Mean (range) herd size	15 (1–200)	12 (1–100)	Not given
Sampled herds			
Number of herds	55	49	40
Herd sizes			
<11	46	27	9
11–50	9	17	20
51–100	0	3	9
>100	0	2	2

DVO, district veterinary officer.

subsequently visited from January to March 2015 by the second author and a team of local veterinary officers and veterinary assistants. A simple random sample of two herds was made from the village list by drawing pieces of paper with farmer names from a bowl. The farmers were visited, informed about the study and asked to participate. In Kamwenge district, due to few local team members and poor road conditions, selection of the first farmer was instead made by driving five minutes from the village centre and asking the nearest farmer with at least two cattle if (s)he was willing to participate and, if not, the next farmer in that direction was asked until two farmers were recruited. In total, one farmer declined to participate.

The teams were informed about the study before recruiting farmers, and further trained before sampling and data collection. Where possible, young animals (5–24 months) were prioritised for sampling to reflect the recent infection history of the herd. Blood samples were kept in a cool box until centrifuged and transferred to a freezer (exact temperature varied but always below the freezing point). Sera were transported to the laboratory at Makerere University in Kampala, where samples were stored in −20°C or −80°C until analysed. During the farm visit, the farmer was also interviewed about management and cattle health.[12] Unless the farmer suggested communication in English, information and interviews were conducted in the local language (Lutoro, Luchiga or Lukonzo).

Serological assays

Serum samples were analysed at Makerere University in Kampala using commercial ELISAs. For CBPP, the IDEXX CBPP *Mycoplasma mycoides* subspecies mycoides antibody test kit (IDEXX Laboratories, Westbrook, USA) was used, for RVF the ID Screen Rift Valley Fever competitive ELISA (IDvet, Grabels, France), and for FMD the Prio-CHECK FMDV NS (Thermo Fisher Scientific, Waltham, USA). The assays were run according to the manufacturers' instructions and using the recommended cut-offs for test interpretation. Samples were randomly assigned to different plates using the random number generator in Microsoft Excel (Microsoft), that is, samples from the same herd were not systematically allocated to the same plate. For each test, the positive and negative controls were compared for all plates to assess if there was any between-plate variation.

Statistical analyses

Data management and statistical analyses were performed in the statistical package R, V.3.2.2 and V.3.2.5.[15] For each disease, a herd was defined as positive if at least one animal had a positive test result. Descriptive statistics were produced and apparent herd prevalence with exact binomial confidence intervals was calculated. The median was used in place of the mean values of all continuous variables that were skewed. For each disease, the interview variables from farmers with positive herds were compared with those from negative herds by the Wilcoxon rank-sum test (continuous) or the Fisher's exact test (categorical).

True prevalence was estimated from a mixed logistic model (by 'glmer(infected~(1|herd),family=binomial)' in R) with herd as random effect to account for clustering. Based on the model, true prevalence of individuals, with 95% CIs, was calculated using the formula:

$$\mathrm{TP} = \frac{\mathrm{AP}-(1-\mathrm{Sp})}{\mathrm{Se}-(1-\mathrm{Sp})}$$

where TP denotes true prevalence, AP denotes apparent prevalence, Se denotes sensitivity and Sp denotes specificity.[16] Herd prevalence was then calculated as:

$$\mathrm{TP}_{\mathrm{Herd}} = 1 - \frac{1}{H}\prod_{i=1}^{H}\left(1 - TP_i\right)^{n_i}$$

where H denotes the number of herds and n_i denotes the number of sampled individuals in herd i. All individual results were thus recalculated, and then true herd level prevalence was calculated. The true herd prevalence hence depends on the actual number of sampled individuals and should be interpreted as an average over all herds. Lower and upper limits of CIs for true prevalence were calculated using lower and upper limits of AP using the same formulas above. Since true prevalence is closely linked to both sensitivity and, in particular, specificity of the ELISA, true prevalence was calculated for sensitivities of 0.8, 0.9, 0.95 and 0.99 and for specificities in the range from 0.95 to 1. In addition, true prevalence was calculated for sensitivity and specificity of the tests according to the most recent reports in the literature.[17–19]

RESULTS

Details of the sampled herds have been reported previously.[12] Herd sizes as assumed before sampling and actual herd sizes of the study herds are shown in Table 1. In total, 138 of the herds had young individuals and were included in the current study. Most herds (71.5 per cent) consisted of up to 20 animals. Serum was collected from a total of 899 young cattle. In 20 herds, fewer cattle than planned were sampled because animals escaped, died, had been sold or could not be handled. The number of analysed samples was lower than intended for RVF and CBPP due to invalid ELISA runs/plates. A problem with the distilled water in the lab was identified and rectified but for practical and economic reasons the plates could not be replaced.

More than 70 per cent of the farmers were satisfied with the health status of their cattle. Notably, more than 30 per cent of the 138 farmers reported abortions and more than 50 per cent reported cattle deaths. Some farmers reported previous CBPP cases and it appeared that most would recognise the typical signs of all three diseases. The median number of abortions and the median number of dead cattle were higher in herds that were test positive for RVF and CBPP ($P<0.01$). In addition, the proportion of herds that had experienced abortions was higher among those test positive for CBPP than among test-negative herds ($P<0.05$).

TABLE 2: Number of tested and seropositive herds and individual cattle from three districts in Western Uganda in 2015. A herd was classified as positive if at least one individual tested positive

	CBPP	RVF	FMD
Tested herds (n)	126	122	138
Tested cattle (n)	640	456	899
Median number of tested cattle per herd (Q1; Q3)*	3 (2; 7)	3 (1; 2.75)	4 (2; 9.75)
Positive herds (n)	38	7	12
Positive cattle (n)	66	9	12
Apparent HP (95% CI)†	30.2 (22.3% to 39.0%)	5.7 (2.3% to 11.5%)	8.7 (4.6% to 14.7%)

*First and third quartiles.
†Apparent herd prevalence with exact binomial CI.
CBPP, contagious bovine pleuropneumonia; FMD, foot-and-mouth disease; RVF, Rift Valley fever.

Apparent prevalence

The number of tested herds and cattle as well as the number of positives and apparent herd prevalence are presented in Table 2. The locations of positive and negative herds are shown in Fig 1a–c. For CBPP, there was a higher proportion (P<0.01) of positive herds in Kasese district (24 of the 38 positive herds). For RVF and FMD, there was no difference between districts. No farmer responded that they had vaccinated against CBPP and no RVF vaccination had reportedly been carried out in the area, but 10 farmers in Kasese district stated that their cattle had been vaccinated against FMD. Two of these farmers each had one test-positive animal. The last vaccination campaign in Kasese was in 2013, and in Kabarole in 2004. In Kamwenge, no vaccination had been carried out for at least seven years.

There was very little between-plate variation for the positive and negative controls of all three tests.

Estimated true prevalence

The calculated true herd prevalence is presented in Fig 2 for all three diseases. Test sensitivity did not affect the true prevalence as much as test specificity. An increase in specificity from 0.95 to 0.99 could result in an increase of 0.01–0.12 in the figures for estimated true herd prevalence (Fig 2).

Using literature estimates of 0.8 sensitivity and 0.98 specificity for the CBPP test[19] yielded a true herd prevalence of 37.0 per cent (95% CI 29.5 to 44.1). A sensitivity of 0.98 and a specificity of 0.99 for the RVF test[18] yielded a true herd prevalence of 3.6 per cent (95% CI 0 to 9.7). Finally, a sensitivity of 0.99 and a specificity of 0.98 for the FMD test[17] yielded a negative estimate (since the AP is less than [1-Sp]).

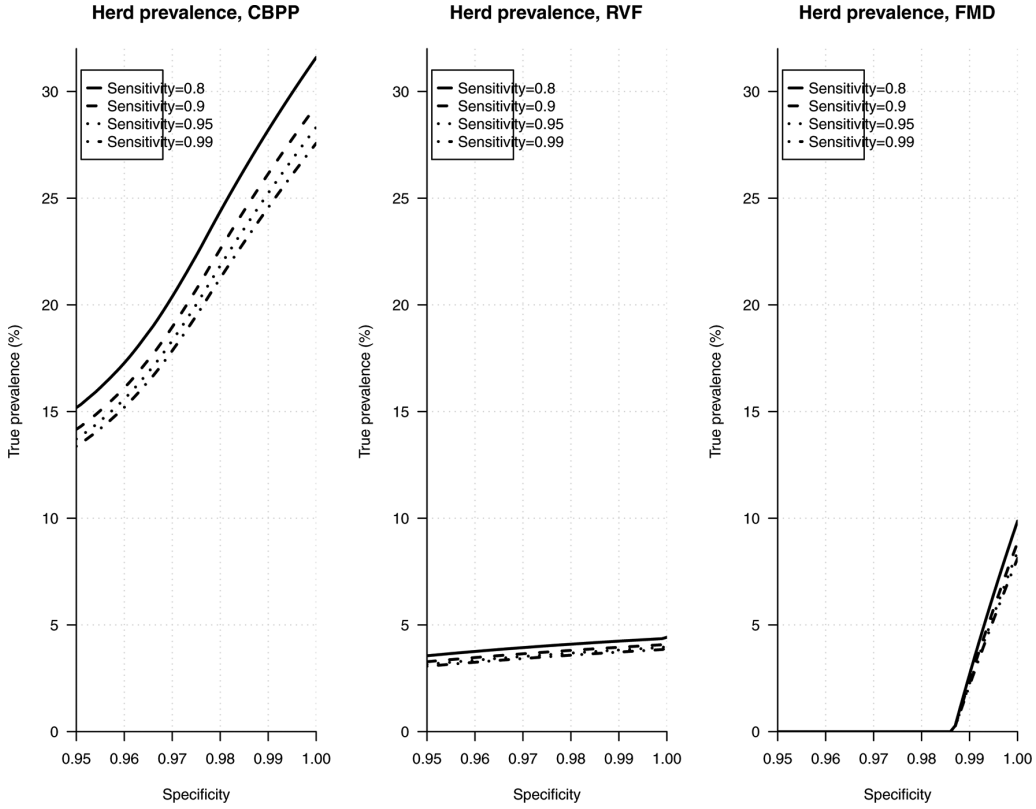

FIG 2: Results from calculations of true herd prevalence based on serological results for contagious bovine pleuropneumonia (CBPP), Rift Valley fever (RVF) and foot-and-mouth disease (FMD) and with varying test sensitivity and specificity.

DISCUSSION

Sampling

In this study, much effort was made to achieve a representative sample but existing infrastructure did not always allow for the planned strategy. Fig 1a–c indicates a fairly even geographic distribution of samples in Kabarole but not in Kamwenge and Kasese. Random or geographic selection of villages was made in all districts to avoid geographic bias. However, assessment of such bias would have required knowledge of the geographic distribution of the entire cattle population and this information was not available. Official statistics on the livestock population are outdated and the DVOs could not provide precise estimates. Moreover, herd size figures are difficult to obtain in the study area due to cultural barriers. Hence, herd size estimates were made on the spot. The farmers were asked about herd size but not for exact figures. Herd size categories based on this estimation are shown in Table 1. Serology reflects historical exposure and the aim was to test young individuals, but as individual identifiers are not generally applied to cattle in the study area, farm records are rare, and animal body sizes depend on nutritional status, it is possible that some animals were incorrectly classified as young. Backward calculation from the actual sample sizes obtained and comparison with the target sample size showed that the reduced sample size resulted in a slightly poorer precision (12 per cent instead of 10 per cent).

Farmer interviews

Good language skills among the local staff permitted communication with the farmers in their local language. However, cultural barriers were found to exist as regards some information, for example, exact herd sizes, that could not be asked for directly.

Most farmers stated that they were satisfied with their animal health status, despite reporting health problems. In a naïve population, the three infections under study would be expected to produce clinical signs and affect herd health. Based on previous experience[12] most farmers in the area perceive some deaths and abortions/reproductive failures as 'normal' and not necessarily a cause for dissatisfaction with herd health. Hence, farmers' estimates of herd health status may not be very useful as an indicator of disease prevalence in passive surveillance.

Disease prevalence

Several farmers stated that they had experienced CBPP in their herd. Presumably, this diagnosis was made based on typical symptoms. CBPP appears to be endemic in Uganda[5 6] and the estimated prevalence (Table 2) would thus appear realistic.

The results could indicate that RVFV is present at low level (Table 2). In theory, some regions in Uganda have conditions that would favour RVF outbreaks as regards climate, landscape, vector population, livestock production and other risk factors.[8] Serological reactions to RVF have been reported in goats[9] and human cases of RVF have been reported after the study period.[10 11] In the light of this, our results most likely indicate the presence of RVFV in the study region.

The NS ELISA detects antibodies to non-structural proteins of the FMD virus and can distinguish between antibodies from vaccination and those from natural infection. However, serological reactions are commonly seen in animals vaccinated with insufficiently purified vaccines. Serological reactions to FMD and isolation of SAT1-type FMD virus from apparently healthy animals have been reported in Kasese district in samples taken in 2011.[20] SAT1 virus has been found in African buffalo,[20] and wildlife from Queen Elizabeth National Park live close to (intermingled on pasture) livestock in Kasese. Four out of the 12 positive herds in the current study were from Kasese. During the period from 2001 to 2008, up to 311 FMD outbreaks were reported in Uganda.[21] It would hence not be unlikely to find seropositive animals in the current study area that had been introduced from another region in Uganda. In our opinion, the results do not indicate any circulation of FMD virus in the sampled herds.

Test characteristics

By using the described sampling and testing strategy, prevalence within the herd cannot be assessed. HSe and HSp become complicated to calculate when the numbers of tested individuals per herd vary, even though the cut-off for positive herd was one positive individual for all herd sizes and all studied diseases. This testing approach is common when many herds of varying size are sampled and all animals cannot be included in the survey. As seen in Fig 2, the sensitivity and specificity of the test are very important for the ability to provide a correct estimate of the true herd prevalence. The test results (AP) lie within the 95% CI of the true prevalence estimates based on reported test characteristics for RVF and CBPP. The negative estimate for FMD suggests that the specificity is higher than the 0.98 estimate from Brocchi *et al*.[17] Although the serological results appear to roughly reflect the true prevalence, the difference between the AP and the estimated true prevalence indicates that they should be interpreted with caution, particularly for FMD. The use of herd-level interpretation and not individual interpretation was based on the expected clustering on herd level of infectious diseases and hence common practice for diagnostic testing for many such diseases. The test manufacturers usually do not provide direct figures for test sensitivity and specificity but, in addition to the more recent studies cited above, refer to some earlier studies for the CBPP and FMD tests.[22–24] However, we elected to use the most recent studies that clearly assess the exact same tests as we used.

In theory, if all FMD test-positive individuals (n=12) were false positives, the specificity for the FMD ELISA would be 887/899=98.7 per cent. If all RVF test-positive individuals (n=9) were false positives, the specificity for the RVF ELISA would be 447/456=98.0 per cent. False

positives are possible for all three tests but, as discussed above, it is still likely that some were true positives.

Technical challenges

Laboratory work in low-income countries brings additional challenges such as frequent power cuts and variations in room temperature that may affect test performance and quality of stored material (samples and test ingredients). National reference laboratories are expected to have quality assurance systems that address these problems but many studies are conducted under more challenging circumstances and, in addition, laboratory quality assurance systems cannot compensate for weaknesses in sampling and/or handling of samples. Transportation of samples is usually necessary, as well-equipped laboratories are rare in rural areas. In this study, we had to rely on cool boxes during transportation and storage of serum in freezers with varying temperature. Such temperature variations may affect sample quality, at least in the long run, but cannot be avoided in most resource-poor settings.

Random allocation of samples to test plates addresses the risk of bias due to differences between test runs. Variation between plates may be an indicator of poor performance or simply an effect of uneven distribution of positive samples between plates. Performance must be checked by comparison of the control samples on the different plates.

Wider implications

The presence of FMD, RVF and CBPP affects livestock export opportunities. Our results highlight the importance of diagnostic test performance when assessing disease status. In theory, calculations of true prevalence should always be included in reports on serosurveys but this is not always the case. In areas that are expected to be free of disease, confirmatory testing of positive samples is usually performed to eliminate false positives. However, this leads to a reduction in overall sensitivity and, consequently, a higher risk of false negatives and premature declaration of freedom. Hence, it is important to include all aspects listed in OIE World Organisation for Animal Health guidelines for declaration of disease-free areas[2] in the assessment. In a situation where the aim is disease eradication, the initial focus is on eliminating false negatives, the concern over false positives is more relevant in the final stages of disease eradication. If the aim is to compare cases and non-cases, for example, for risk factor studies, test interpretation and case definition become even more important. As calculations of true prevalence cannot compensate for misclassification of cases and non-cases, risk factor studies should ideally use more than one criterion for case definition. This may be in the form of combining results from different or repeated tests and/or clinical assessment.

In conclusion, test characteristics are crucial for the interpretation of results from serosurveys, particularly in resource-poor settings where additional challenges may affect test performance. Calculations that take these factors into account should be applied to a larger extent in such studies and case definitions should be based on more than a single serological result. Moreover, sampling should, as far as possible, be based on sample size calculations. In the absence of animal registers, sampling frames should be created manually in real time. All these aspects should be addressed and elaborated when publishing data from serosurveys, especially in resource-poor settings.

Definitions

Individual prevalence=number of positive individuals/number of sampled individuals.

Herd prevalence=number of positive herds/number of sampled herds.

Apparent prevalence=number of test positive/number of sampled.

True prevalence=number of truly positive/number of sampled.

Seroprevalence=prevalence figures based on serological results (reflecting seroconversion).

Test sensitivity=number of test-positive samples/number of truly positive samples.

Test specificity=number of test-negative samples/number of truly negative samples.

Herd sensitivity=number of test-positive herds/number of truly positive herds.

Herd specificity=number of test-negative herds/number of truly negative herds.

ETHICAL ASPECTS

Informed consent was given (orally) by all participants after they were informed about the study and that their identities were not to be included in any reports. Samples were taken under the direct supervision and mandate of the DVOs (Ugandan Animal Disease Act, Chapter 38, part III, point 9). Any farmer who declined to participate at recruitment or had a change of mind at the farm visit was removed from the study. It was stressed to the local team that participation was voluntary and farmers should not be forced or persuaded to participate. The results from the study have been shared and discussed with the DVOs and representatives from the Ugandan Ministry of Agriculture, Animal Industry and Fisheries.[13]

Acknowledgements The authors thank the DVOs Drs Salvatory Abigaba, Alfred Kamanyire and Kalule Godfrey and their respective teams for dedicated assistance with the fieldwork, and the participating farmers for generously sharing their time and experiences. We also thank Mr Johnson Mayega, and Drs Anna and Sofia Cavalli-Björkman Hellström for diligent laboratory work.

Contributors CW and SSL designed the study with support from KS and SB. CW planned and carried out the fieldwork. MAF carried out the statistical analyses. CM was responsible for the laboratory work. SSL drafted the manuscript. All authors revised the manuscript and approved the final version.

Funding This work was supported by the Swedish Research Council (Vetenskapsrådet) (grant number 348-2013-6608).

Competing interests None declared.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement There are no additional unpublished data.

REFERENCES

1. Rich KM, Perry BD. The economic and poverty impacts of animal diseases in developing countries: new roles, new demands for economics and epidemiology. *Prev Vet Med* 2011;101:133–47.
2. OIE Code. Terrestrial Animal Health Code. Chapter 1.6 Procedures for self declaration and for official recognition by the OIE.. http://www.oie.int/index.php?id=169&L=0&htmfile=chapitre_selfdeclaration.htm (accessed 25 Oct 2017).
3. Balinda SN, Tjørnehøj K, Muwanika VB, *et al*. Prevalence estimates of antibodies towards foot-and-mouth disease virus in small ruminants in Uganda. *Transbound Emerg Dis* 2009;56:362–71.
4. Kasambula L, Belsham GJ, Siegismund HR, *et al*. Serotype identification and VP1 coding sequence analysis of foot-and-mouth disease viruses from outbreaks in eastern and northern Uganda in 2008/9. *Transbound Emerg Dis* 2012;59:323–30.
5. Baluka SA, Hisali E, Wasswa F, *et al*. Socio-economic risk factors associated with foot and mouth disease, and contagious bovine pleuropneumonia outbreaks in Uganda. *Livestock Res. Rural Dev* 2013;25:12.
6. OIE WAHISa. Animal Health Information System, detailed disease information by country. http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/statusdetail (accessed 25 Oct 2017).
7. OIE WAHISb. Animal Health Information System, vaccination. http://www.oie.int/wahis_2/public/wahid.php/Countryinformation/Vaccination.
8. Tran A, Trevennec C, Lutwama J, *et al*. Development and assessment of a geographic knowledge-based model for mapping suitable areas for rift valley fever transmission in Eastern Africa. *PLoS Negl Trop Dis* 2016;10:e0004999.
9. Magona JW, Galiwango T, Walubengo J, *et al*. Rift Valley fever in Uganda: seroprevalence and risk factor surveillance vis-à-vis mosquito vectors, anti-RVF virus IgG and RVF virus neutralizing antibodies in goats. *Small Rum Res* 2013;114:176–81.
10. De St. Maurice A, Nyakarahuka L, Purpura L, *et al*. Rift Valley fever response – Kabale district, Uganda, March 2016. CDC Morb. *Mortal. Wkly Rep* 2016;65:1200–1.
11. Ministry of Health, 2017. Press realease: Rift Valley fever outbreak confirmed in Mityana and Kiboga districts. https://reliefweb.int/sites/reliefweb.int/files/resources/Press%20Release%20-%20RVF%20OUTBREAK.pdf (accessed 6 Dec 2017).
12. Wolff C, Boqvist S, Ståhl K, *et al*. Biosecurity aspects of cattle production in Western Uganda, and associations with seroprevalence of brucellosis, salmonellosis and bovine viral diarrhoea. *BMC Vet Res* 2017;13:382.
13. Ministry of Agriculture, Animal Industry and Fisheries (MAAIF) & Uganda Bureau of Statistics (UBOS) 2009. The national livestock census report, 2008. file:///Users/susl/Downloads/NLC2008%20Final%20Report.pdf. (accessed 25 Oct 2017).
14. Wensman JJ, Lindahl J, Wachtmeister N, *et al*. A study of Rift Valley fever virus in Morogoro and Arusha regions of Tanzania - serology and farmers' perceptions. *Infect Ecol Epidemiol* 2015;5:30025.
15. Core Team R. *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing, 2016. https://www.R-project.org/. (accessed 25 Oct 2017).
16. Christensen J, Gardner IA. Herd-level interpretation of test results for epidemiologic studies of animal diseases. *Prev Vet Med* 2000;45:83–106.
17. Brocchi E, Bergmann IE, Dekker A, *et al*. Comparative evaluation of six ELISAs for the detection of antibodies to the non-structural proteins of foot-and-mouth disease virus. *Vaccine* 2006;24:6966–79.
18. Kortekaas J, Kant J, Vloet R, *et al*. European ring trial to evaluate ELISAs for the diagnosis of infection with Rift Valley fever virus. *J Virol Methods* 2013;187:177–81.
19. Sidibé CA, Grosbois V, Thiaucourt F, *et al*. Performance evaluation of two serological tests for contagious bovine pleuropneumonia (CBPP) detection in an enzootic area using a Bayesian framework. *Trop Anim Health Prod* 2012;44:1233–8.
20. Dhikusooka MT, Ayebazibwe C, Namatovu A, *et al*. Unrecognized circulation of SAT 1 foot-and-mouth disease virus in cattle herds around Queen Elizabeth National Park in Uganda. *BMC Vet Res* 2016;12:5.
21. Ayebazibwe C, Tjørnehøj K, Mwiine FN, *et al*. Patterns, risk factors and characteristics of reported and perceived foot-and-mouth disease (FMD) in Uganda. *Trop Anim Health Prod* 2010;42:1547–59.
22. Sørensen KJ, Madsen KG, Madsen ES, *et al*. Differentiation of infection from vaccination in foot-and-mouth disease by the detection of antibodies to the non-structural proteins 3D, 3AB and 3ABC in ELISA using antigens expressed in baculovirus. *Arch Virol* 1998;143:1461–76.
23. CIRAD/Pourquier. Serological detection of specific antibodies to *Mycoplasma mycoides* subspecies *mycoides* Small Colony (MmmSC), the etiologic agent of contagious bovine pleuropneumonia (CBPP). 2002 https://ca.idexx.com/pdf/en_ca/livestock-poultry/cbpp-ab-test-insert.pdf (accesed 11 Dec 2017).
24. Schubert E, Sachse K, Jores J, *et al*. Serological testing of cattle experimentally infected with Mycoplasma mycoides subsp. mycoides Small Colony using four different tests reveals a variety of seroconversion patterns. *BMC Vet Res* 2011;7:72.