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Short Communication

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# The use of Immunodiagnostic Techniques in Sheep for the Epidemiological Surveillance of Cystic Echinococcosis

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**ABSTRACT**

**Objective:** Cystic echinococcosis (CE) is a parasitic zoonosis caused by *Echinococcus granulosus sensu lato.* Immunodiagnostic techniques such as Western blot (WB) or enzyme-linked immunosorbent assay (ELISA), with different antigens, can be applied to the diagnosis of sheep for epidemiological surveillance purposes in control programs. However, its use is limited by the existence of antigenic cross-reactivity between different species of taeniidae present in sheep. Therefore, the usefulness of establishing surveillance systems based on the identification of infection present in a livestock establishment, known as the (Epidemiological) Implementation Unit (IU), needs to be evaluated.
**Materials and Methods:** A new ELISA diagnostic technique has been recently developed and validated using the recombinant EgAgB8/2 antigen for the detection of antibodies against *E. granulosus*. To determine detection of infection at the IU level using information from this diagnostic technique, simulations were carried out to evaluate the sample size required to classify IUs as likely infected, using outputs from a recently developed Bayesian latent class analysis model.
**Results:** Relatively small samples sizes (between 14-29) are sufficient to achieve a high probability of detection (above 80%), across a range of prevalence, with the recently recommended Optical Density cut-off value for this novel ELISA (0.496), which optimizes diagnostic sensitivity and specificity.
**Conclusions:** This diagnostic technique could be potentially used to identify the prevalence of infection in an area under control, measured as the percentage of IUs with the presence of infected sheep (infection present), or to individually identify the IU with ongoing transmission, given the presence of infected lambs, on which control measures should be intensified.

**Keywords:** Echinococcosis, immunodiagnostics, sheep, surveillance, control

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## INTRODUCTION

Cystic echinococcosis (CE) is a parasitic zoonosis caused by *Echinococcus granulosus sensu lato*, a cestoda parasite in the Taeniidae family. The life cycle involves two mammalian hosts. The definitive ones are carnivores (especially dogs), and the intermediate ones are ungulates (being sheep and goats the ones of greater epidemiological importance in many parts of the world) (1).

The surveillance of CE in the framework of a control programme is directed towards the different hosts: mainly humans (who are accidental hosts), dogs, sheep and goats (2). Diagnosis in sheep and goats can be made macroscopically at slaughter (via necropsies) (3-5). However, in many endemic areas, slaughterhouses are rare: the urban supply of meat usually originates from local butchers without any type of sanitary infrastructure or veterinary inspection; in rural areas on the other hand, home slaughter for personal consumption or retail sale is the norm. Therefore, while post-mortem inspection is the technique of choice, it is challenging to support a surveillance system on this data source6. Moreover, macroscopic diagnosis in lambs also has limitations, in particular false negatives from recent infections that may not be detected, or from newly formed small hydatid cysts that are unlikely to be observed. In older animals, false positives are also possible from the presence of degenerated or calcified cysts due to other infections or conditions (4).

Immunodiagnostic techniques such as western blot (WB) or enzyme-linked immunosorbent assay (ELISA), with different antigens, can be applied to the diagnosis of ovine CE (3,5,7,8). However, there is a limitation for its use in sheep, as infections by parasites other than *E. granulosus (T. hydatigena*, *Monezia*, *Tænia ovis*, *Tænia multiceps)* are common in sheep and goats, and there is evidence of antigenic cross-reactivity between different species of taeniidae (2,5,7).

Here, we discuss a CE surveillance strategy focusing on the implementation of sheep serology at an epidemiological unit level (IU), such as a livestock farm, rather than at individual level, that takes advantage of a novel recombinant antigen for ELISA

## MATERIALS AND METHODS

### Immunodiagnosis in sheep

The survival of *E. granulosus* depends on efficient evasion mechanisms that operate towards the development of a hydatid cyst (1,2,7). Thus, the development of immunodiagnostic tests for screening aimed at the detection of *E. granulosus* depends on the interaction between the host and the parasite during the infection.

The immune response of the ruminant host against infection is directed towards the oncosphere, components of the immature cyst and/or fertile metacestodes and protoscolices (1,2,7). An IgG response to the fluid of the hydatid cyst of *E. granulosus*, i.e. antigens in the oncosphere, appears between four to eleven days post-infection in sheep experimentally challenged with either eggs or oncospheres, and persists for at least 4 years. However, it does not always lead to a significant increase in antibody titres and is also not maintained throughout the course of infection (9,10).

The hydatid fluid is a complex mixture of different antigens, the main ones being the antigenic lipoproteins: Ag 5 and Ag B. Ag B is the most abundant and is a thermostable lipoprotein of 120-160-kDa containing subunits: 8 or 12, 16, and 20 or 24 kDa (1,2,6). A multigenic family coding for the 8-kDa antigen (EgAgB8/1 to EgAgB8/5) was found to be composed of many members with high diversity, so its use can provide molecular evidence of cross-reaction, or specific reaction, for infections with metacestodes (1,2,6,11). A new ELISA diagnostic technique has been recently developed and validated using the recombinant EgAgB8/2 antigen for the detection of antibodies against *E. granulosus* (11).

To collect the samples for the ELISA in sheep, 10ml of blood from the jugular vein can be drawn by holding the animal in a standing position with their heads fixed laterally, using 25/8 needles and disposable plastic syringes. The samples need to be labelled with one number identifying the animal and another identifying the producer. Ideally the data will be collated in a registration form that contains the numbers cited, the name of the producer, the geographical area (or otherwise be geo-referenced) and the date of sampling. It is also beneficial to record the total number of existing sheep and lambs, which can facilitate further analysis. Serum can be extracted by centrifugation and must be kept refrigerated at 5 to 8ºC until its referral to the laboratory (48 hours maximum), where it can be kept in a freezer at – 20ºC until it is processed.

### Sample size estimation

To determine a suitable sample size for the evaluation of infection at the IU level, outputs of a Bayesian Latent Class Analysis framework developed by Sykes et al., implementing a Markov chain Monte Carlo algorithm (12) were used. Briefly, the model infers the 'true' infection status of individual sheep based on multiple diagnostic techniques, without assumptions about a gold standard. In the work by Sykes et al., the model used data from necropsies, the recombinant EgAgB8/2 antigen and western blots from 79 adult sheep.

Extending on this work, posteriors drawn from the Bayesian model were used to simulate IUs for a range of infection

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prevalence and to evaluate different sample sizes for high probabilities of CE detection. Nine farm scenarios were simulated, with prevalence in the farm of 1%, and 5% to 40% (in 5% increments). The deployment of the ELISA diagnostic technique was simulated, with different sample sizes from 1 to 100 sheep taken in each simulated farm. The posterior distribution for the sensitivity and specificity was used for the ELISA technique with an optical density cut-off value of 0.496, as defined in Sykes et al. (12). The lowest sample size needed for probabilities of detection of 80% and 90% was calculated. This can be defined as the proportion of IUs correctly identified as infected (with prevalence >0%). An IU was assumed to be infected if two or more samples came back positive (12).

## RESULTS

The specificity and analytical sensitivity of the ELISA diagnostic technique were evaluated with a panel of control sera of experimentally infected sheep (n=40), free of the disease (n=79), and animals naturally infected with other parasites (n=20), observing a satisfactory capacity of discrimination between positive sera of different reactivity, negative sera, and laboratory controls without antigen (11). The performance of this ELISA diagnostic at both the individual level and at the herd level was determined by ROC curves, estimating an optical density of 0.496 as an appropriate cut-off value that optimizes sensitivity and specificity at the IU level (12).

**Table 1: Reference for estimation of sample sizes according to the expected prevalence to identify transmission present with a cut-off value of 0.496, depending on the desired probability of detection. For an expected prevalence greater than 40%, it is recommended to use the sample size estimated at 40%**

| Probability of detection | Expected Prevalence | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1% | 5% | 10% | 15% | 20% | 25% | 30% | 35% | 40% |
| **80%** | 21 | 18 | 18 | 17 | 16 | 16 | 15 | 14 | 14 |
| **90%** | 29 | 28 | 25 | 22 | 21 | 20 | 20 | 18 | 18 |

The sample size required at the IU level will change depending on the minimum probability of detection wanted for the programme, Table 1. Achieving a higher probability of detection requires larger sample sizes. It is important to highlight that the number of animals in the herd has a negligible effect on the sample size in these settings where IUs have hundreds (or even thousands) of animals.

## DISCUSSION

Traditionally, CE surveillance has been based on estimating the percentage of parasitized dogs (13). An alternative approach would be to establish surveillance systems based on the identification of infection present in a livestock establishment (i.e. a farm), here our IUs, based on an assessment of the sheep population. This has the advantage that ongoing transmission in IUs could be assessed based on the presence of lambs infected with *E. granulosus*, while presence of CE, which might not imply current ongoing transmission, could be evaluated from infections in older animals. Moreover, CE could be identified in herds that are scheduled to enter areas free of infection. Furthermore, the effectiveness of remedial actions could more readily be inferred, where transmission might be maintained despite control measures in place, thus requiring the intensification of activities. Baselines and trends of CE in the area under a programme could be equally evaluated from the percentage of IUs with infected animals, supporting the evidence base for future activities.

It is important to highlight that CE control programmes generally have no interest in the individual diagnosis of *E. granulosus* infection, since there is a lack of a validated specific treatment. The main objective of the programme remains the determination of infection in the herd. To use immunodiagnosis in sheep for surveillance purposes, two requirements need to be met: 1) A validated serological technique needs to be available; and 2) it should be feasible to collect a suitable, representative, sample size. The sample size needs to be appropriate to the objective, minimizing the bias given by cross-reaction with other cestodes.

As mentioned above, the recombinant EgAgB8/2 antigen recently validated could be a suitable serological technique, while, as shown in Table 1, the sample sizes required for IU are potentially feasible in many settings. Prevalence in the IU is generally not known, but the sample size needed will depend on it, therefore an “expected” prevalence needs to be assumed. This value can be inferred from knowledge of the local epidemiology and bibliographic background or reports from comparable studies (14). While a detection of 90% (or higher) would be recommended, in many resource-constrained settings 80% would be sufficient. Moreover, while an epidemiological implementation unit would generally consist of a single livestock establishment with enough animals to cover the sample, in the case of small producers with few animals, the IU sample may be drawn among several producers, particularly in the case

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of indigenous reserves and communal grounds that are shared as grazing pastures. The appropriate sample will then be selected by randomly choosing animals, which can prove more cost-effective.

While there are no field studies published yet using this approach, there are a few ongoing. A study in the northern region of San Luis province in Argentina is evaluating control in sheep and goats using the commercial vaccine Providean Hidatec EG95®, and will evaluate the recombinant EgAgB8/2 antigen in these two species, as well as another diagnostic in dogs (copro-antigen)15. Another study in the province of Misiones, Argentina is directly evaluating the recombinant EgAgB8/2 antigen in the field, in both sheep and goats (16).

## CONCLUSION

Modern CE surveillance systems generally have one of two aims: either 1) characterize the prevalence of infection in an area under control measured by the proportion of IUs with infected sheep, which can be used to monitor progress of different interventions; or 2) identify IUs with ongoing transmission by investigating infection in young lambs, which again may lead to intensification of control measures such as deworming or vaccination.

To use immunodiagnosis in sheep for surveillance purposes, a validated serological technique needs to be available with an appropriate sampling design. Therefore, the ELISA in sheep described above can be used, alone or associated with the traditional CoproELISA in dogs (2,9), or other diagnostic techniques in the definitive host, as a new tool for monitoring CE in a standardized way for control and surveillance programmes. Logistically, whether it is sheep or dog samples, both are obtained by the same personnel, therefore both approaches could be used synergistically, as they are simple, economical and accessible to countries with limited resources and laboratory capacity. These would lead to enhanced surveillance of *E. granulosus* transmission and better evidence to adjust control measures.

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