Veterinary Parasitology 203 (2014) 59–64

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Ultrasound as a monitoring tool for cystic echinococcosis in sheep

F. Dore[a], A. Varcasia[a,*], A.P. Pipia[a], G. Sanna[a], M.L. Pinna Parpaglia[a], A. Corda[a], T. Romig[b], A. Scala[a]

[a] Parasitology and Parasitic Diseases, Department of Veterinary Medicine of Sassari, University of Sassari, Italy
[b] Universität Hohenheim, FG Parasitologie, Stuttgart, Germany

ARTICLE INFO

Article history:
Received 15 January 2014
Received in revised form 6 March 2014
Accepted 12 March 2014

Keywords:
Cystic echinococcosis
Sheep
Ultrasonography
Intra vitam diagnosis
Sardinia

ABSTRACT

An ultrasound-based survey for cystic echinococcosis (CE) in sheep was carried out in Sardinia in 2012. The study was done on three farms (A, B, C) which had been pre-selected for different CE prevalence levels (A: >80%, B: 50–80%, C: <50%). In total, 129 sheep were examined on the farms using portable ultrasound equipment (A: $n = 51$, B: $n = 30$, C: $n = 48$). Within a period of 20 days after ultrasound examination, all sheep were slaughtered and underwent a parasitological post-mortem examination for cysts in the liver and lungs. With post-mortem as gold standard, ultrasonography gave a test sensitivity of 88.7% and a specificity of 75.9%, while the positive and negative predictive values were 81.8% and 84.6%, respectively. When only sheep with fertile cysts were considered, the sensitivity of the test increased to 100%. We conclude that the ultrasound examination of the liver in sheep – using state-of-the-art technology – is a sensitive and specific diagnostic tool, which is cost-effective, highly appropriate for field use and requires only moderate time (no shaving required). The method can also be applied to other livestock species and will be useful tool in epidemiological studies, monitoring schemes and vaccination/control trials.

1. Introduction

Cystic echinococcosis (CE), a disease of humans and livestock, is caused by metacestodes of *Echinococcus granulosus* sensu lato. It is considered as one of the most important zoonoses worldwide (Eckert and Deplazes, 2004) and as an emerging/re-emerging disease (Jenkins et al., 2005; Buishi et al., 2005).

Factors that favour CE persistence include extensive livestock husbandry, presence of numerous stray or shepherd dogs, unsupervised home slaughter and improper disposal of carcasses (Varcasia et al., 2004; Scala et al., 2006; Scala and Mazzette, 2009; Varcasia et al., 2011). All of these risk factors are present on Sardinia, which makes the island one of the regions with the highest endemicity levels in Europe.

In recent years, major advances have been made in the development of recombinant vaccines for the control of metacestode infections (*Taenia* sp., *Echinococcus* sp.) in sheep (Lightowlers, 2006; Lightowlers et al., 2003; Varcasia et al., 2009). A WHO-led project that includes this kind of prophylaxis is being considered for Sardinia in context of the "WHO Global Plan to combat Neglected Tropical Diseases (NTDs)" (Meslin and Magnino, 2012; Lightowlers, 2012). One of the difficulties in planning CE control activities is the lack of a simple, non-invasive method for the diagnosis of CE in sheep. Such a method, however, is of

* Corresponding author. Tel.: +39 079 229467; fax: +39 079 229464.
E-mail addresses: varcasia@uniss.it, antonio@varcasia.com (A. Varcasia).

http://dx.doi.org/10.1016/j.vetpar.2014.03.016
0304-4017/

crucial importance for monitoring the infestation levels in livestock, e.g. as baseline information on the infection pressure in a chosen area, or to evaluate the efficacy of a vaccine or other control measures (Jenkins et al., 2005).

Ultrasonography has already been evaluated as an intra vitam screening tool for CE in sheep (Maxson et al., 1996; Guarnera et al., 2001; Lahmar et al., 2007). However, the recent advances in imaging technology have led to a drastic improvement of the performance of portable ultrasound, e.g. by allowing to monitor the full liver parenchyma in live animals with a microconvex transducer.

The aim of this study was to evaluate portable ultrasound equipment of the latest generation as an intra vitam screening tool for ovine CE of naturally infected animals under field conditions.

2. Materials and methods

2.1. Study area and animal population

The study was conducted from February to December 2012, in three different areas of Sardinia, Italy. The areas were chosen for different CE prevalence levels, which was assessed by post-mortem examination of slaughtered animals prior to the study (Scala unpublished data 2012). In each area, one farm was selected. Expected prevalences were >80% (farm A, in the municipality of Nule, Sassari), between 50 and 80% (farm B, in the municipality of Sassari), and <50% (farm C, in the municipality of Monastir, Cagliari).

In each farm, female sheep of Sarda breed were randomly selected. All sheep included in the trial were born and bred on their farms of origin. The total number of sheep was 129 (farm A: $n = 51$, farm B: $n = 30$, farm C: $n = 48$), the age range was 2.5–13 years (mean: 6.5).

2.2. The ultrasound examinations

Equipment was a portable Logiq® eVet ultrasound (General Electric Company Fairfield, CT, USA) with a microconvex 8 C-RS multifrequency transducer (4–11 MHz). The ultrasound examinations were carried out on site, with sheep in standing position without sedation. Before application of the ultrasound gel, grease and dirt were removed from the fleece with a solution of denatured alcohol and water. The animals were not shaved.

The liver was examined by transverse and longitudinal scans, the acoustic window was the most cranial part of the right hypochondrium, caudally to the last rib. When it was difficult to visualize the liver due to presence of gas in the gastro-intestinal tract, the acoustic window was shifted to the last intercostal space. The liver lesions (cysts) were clearly identified and located. In doubtful cases, colour Doppler was used to differentiate cystic lesions from blood vessels.

On average, the entire procedure (preparation and scan) with each sheep was completed within five minutes. For each lesion, location [right lobe (LD), left lobe (LS), caudate lobe (LC), quadrate lobe (LQ) and hilum (I.)], condition and size were recorded in a form.

The condition (sonographic appearance) of each cyst was identified in accordance with the manual of the World Health Organization (1996) as active cysts (CL, CE1, CE2), transition cysts (CE3) and inactive cyst (CE4, CE5).

2.3. Parasitological post-mortem examination

Within a period of 20 days after the ultrasound examination, all sheep were slaughtered at local abattoirs. Liver and lungs of each animal were palpated and dissected in <1 cm strips in order to identify cysts.

Echinococcus cysts were differentiated from other parasites forming cyst-like structures (*Taenia hydatigena* metacestodes, and distended bile ducts caused by the liver flukes *Fasciola hepatica* and *Dicrocoelium dendriticum*) by macroscopic examination and, if necessary, light microscopy. *Echinococcus* cysts were counted and allocated to their respective ultrasound class (CL, CE1–5 – World Health Organization, 1996), and size category ($\leq$1–3 cm, $\leq$3–5 cm, $\leq$5–10 cm, $\leq$10 cm).

Cysts were further examined for fertility (presence and motility of protoscoleces) using a light microscope without staining.

Abundance (mean number of cysts in examined sheep) and the average intensity (mean number of cysts in infected sheep) were calculated as reported by Bush et al. (2007). Statistical analysis was performed with the software Minitab v. 15.

3. Results

The results of the ultrasound examination are summarized in Table 1. The overall ultrasound prevalence of liver CE was 59.7% (77/129). A total of 344 cysts were found, of which 12 were considered active (Figs. 1–3) and 332 transitional or inactive (Figs. 4–6). Abundance (A) and average intensity (AI) were 2.66 and 4.46, respectively. As expected, CE prevalences differed significantly among the three farms (χ^2 with two degrees of freedom = 28.95, $P < 0.0001$). The ultrasound allowed detection of cysts with a minimum

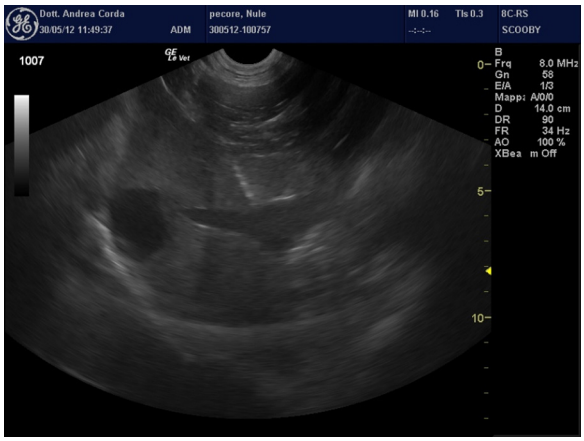

Fig. 1. CL, unilocular, cystic lesion with uniform anechoic content, not clearly delimited by an hyperechoic rim (=cyst wall not visible). Status: active.