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Echinococcus granulosus: Epidemiology and state-of-the-art of diagnostics in animals

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ABSTRACT

Diagnosis and detection of *Echinococcus granulosus* (sensu lato) infection in animals is a prerequisite for epidemiological studies and surveillance of echinococcosis in endemic, re-emergent or emergent transmission zones. Advances in diagnostic approaches for definitive hosts and livestock, however, have not progressed equally over the last 20 years. Development of laboratory based diagnostics for canids using coproantigen ELISA and also coproPCR, have had a huge impact on epidemiological studies and more recently on surveillance during hydatid control programmes. In contrast, diagnosis of cystic echinococcosis (CE) in livestock still relies largely on conventional post-mortem inspection, despite a relatively low diagnostic sensitivity especially in early infections, as current serodiagnostics do not provide a sufficiently specific and sensitive practical pre-mortem alternative. As a result, testing of dog faecal samples by coproantigen ELISA, often combined with mass ultrasound screening programmes for human CE, has been the preferred approach for monitoring and surveillance in resource-poor endemic areas and during control schemes. In this article we review the current options and approaches for diagnosis of *E. granulosus* infection in definitive and animal intermediate hosts (including applications in non-domesticated species) and make conclusions and recommendations for further improvements in diagnosis for use in epidemiological studies and surveillance schemes.

1. Introduction

The genus *Echinococcus* (Family: Taeniidae) has been the subject of several taxonomic revisions since the 1960s. *Echinococcus granulosus* previously comprised up to 9 sub-specific genotypes (G1–G9) or strains, which develop in the larval (hydatid) stage as cystic echinococcosis (CE) in ungulates or other herbivores. The current view informed by biology, epidemiology and particularly molecular genotyping recommends the inclusion of at least 9 species in the genus. All those species of *Echinococcus* known to cause CE in the intermediate host may be referred to as *E. granulosus* sensu lato (s.l.), whereas strains G1–G3 (which are closely related) are now referred to as *E. granulosus* sensu strictu (s.s.) (Nakao et al., 2013; Romig et al., 2015, this issue). The global public health impact of human CE is significant and is caused primarily by the G1 genotype (Budke et al., 2006). Other zoonotic species of *E. granulosus* s.l. include *Echinococcus ortleppi* (G5) and *Echinococcus canadensis* (G6–9) (Alvares Rojas et al., 2014); the zoonotic status of *Echinococcus equinus* (G4) appears unlikely (McManus and Thompson, 2003), and that of *Echinococcus felidis* remains unknown (Huttner et al., 2008).

Diagnosis of echinococcosis in animals is primarily concerned with infections in dog and sheep hosts. The domestic dog is the key definitive host for *E. granulosus* s.s. and thus the main source of human CE worldwide. Dogs also appear to be highly susceptible to all genotypes of *E. granulosus*, and may exhibit different pre-patent periods (Carmena and Cardona, 2013). Wild canids (e.g. *Canis lupus, Canis aureus, Vulpes vulpes*) also show a range of susceptibilities (Jenkins and Macpherson, 2003; Rausch, 2003; Lahmar et al., 2009). Sheep (and goats) are the most important domestic intermediate host for *E. granulosus* s.s. G1, and this genotype itself may also infect other herbivore hosts (e.g. cattle, camels, donkeys and macropods) (Jenkins, 2006; Boufana et al., 2014). Small ruminants are also susceptible to other *Echinococcus* species or genotypes, for example *E. canadensis* (G6) in goats (Soriano et al., 2010). A wide range of other domestic livestock hosts are susceptible to CE and/or involved in transmission of *E. granulosus* s.l. and include cattle, yak, buffalo, camelids, pigs and equids (Eckert et al., 2001).

In this review, we have attempted to update progress in diagnostics for animal echinococcosis due to *E. granulosus* (s.l.) particularly in relation to epidemiological and surveillance applications in domestic animals. We have also included consideration

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of echinococcosis in wildlife and of optimal approaches for analysis/interpretation of epidemiological data. Previous comprehensive reviews on diagnostics in animals include Craig (1997), Zhang and McManus (2006), and Carmena and Cardona (2013).

2. Epidemiological considerations: *Echinococcus granulosus* (sensu lato)

2.1. Risk factors for echinococcosis in domestic animals

A large number of studies have been conducted investigating risk factors for infection of intermediate and definitive animal hosts with *E. granulosus*; many of which have been recently reviewed (Otero-Abad and Torgerson, 2013). In the case of canine infection, a variety of different methods have been used for identification of canine infection—with some measuring coproantigen positivity, some identifying worms following arecoline purgation, and some attempting to adjust these estimates (for example, by accounting for test sensitivity and specificity) in order to estimate the true infection status. Risk factors for canine infection can be grouped into a number of broad categories according to the general suspected process in place (Otero-Abad and Torgerson, 2013). Based upon this classification, access to infected offal appears to be one of the most commonly identified risk factors for canine infection with *E. granulosus*—whether it be due to purposeful feeding of offal/home slaughtering (Carmona et al., 1998; Moro et al., 1999; Buishi et al., 2006; Acosta-Jamett et al., 2010a); lack of restraint/free roaming (Buishi et al., 2005a; Buishi et al., 2006; Guzel et al., 2008; Huang et al., 2008; Mastin et al., 2011); proximity to possible infected offal (Bchir et al., 1987; Wang et al., 2001; Elshazly et al., 2007; Acosta-Jamett et al., 2010a); or dog type (farm/working dogs and stray dogs frequently have a higher probability of positivity) (Moro et al., 1999; Shaikenov et al., 2003; Buishi et al., 2006; Inangolet et al., 2010). A number of studies have also found that older dogs had a lower probability of positivity than younger dogs (Sharifi and Zia-Ali, 1996; Buishi et al., 2005a; Buishi et al., 2006; Acosta-Jamett et al., 2010a; Inangolet et al., 2010), which may suggest some degree of acquired immunity, or may indicate age-related variation in dog behaviour or management (Torgerson et al., 2003b; Torgerson, 2006a). A lack of knowledge about echinococcosis and a lack of recent praziquantel dosing have also been identified as associated with an increased probability of positivity (Buishi et al., 2005a; Buishi et al., 2005b; Huang et al., 2008; Acosta-Jamett et al., 2010a).

In the case of risk factor studies for intermediate host infection, diagnosis is most commonly achieved at necropsy, and may be interpreted in a dichotomous fashion (cysts either present or absent), or with an estimate of the total number of cysts. In some cases, methods such as ultrasonographic examination have been used to classify the infection status of intermediate hosts for risk factor studies (Lahmar et al., 2007a) and cysts may also be classified according to fertility status. Whilst infection in intermediate hosts is a good measure of the level of environmental contamination with eggs, the persistence of cysts following infection means that the current exposures may differ from those present at the time of infection. A recent study of risk factors for intermediate host infection classified these broadly at the environmental, production system and animal level (Otero-Abad and Torgerson, 2013). Most 'environmental factors' were due to spatial variation in the prevalence of infection (Ahmadi, 2005; Azlaf and Dakkak, 2006; Banks et al., 2006; Lahmar et al., 2007a; Acosta-Jamett et al., 2010b; Manfredi et al., 2013), although seasonal (Ansari-Lari, 2005; Daryani et al., 2007; Ibrahim, 2010), climatic (Acosta-Jamett et al., 2010b) and geographic (Fromsa and Jobre, 2011) effects have also been identified in some studies (although some of these conclusions are based on univariable analysis and therefore some apparent associations may be due to confounding). Extensive management systems have also been found to be associated with porcine CE infection (Sharma et al., 2004; Bruzinskaite et al., 2009).

The most consistent risk factor for intermediate host infection and hydatid cyst burden is animal age, with older animals being more likely to be infected and generally harbouring more protoscolices than younger animals (Cabrera et al., 1995; Banks et al., 2006; Scala et al., 2006; Lahmar et al., 2007a; Christodoulopoulos et al., 2008; Bruzinskaite et al., 2009; Torgerson et al., 2009; Ibrahim, 2010; Zewdu et al., 2010; Marshet et al., 2011). This is expected as cyst persistence is generally lifelong—and as such cyst burden represents an ongoing infection pressure over time. Associations with animal sex are variable, with some studies finding no difference and others finding that female animals were more likely to be infected than males (Ming et al., 1992; Daryani et al., 2007; Ibrahim, 2010).

2.2. Endemic and emergent situations for cystic echinococcosis

Cystic echinococcosis in domesticated animals and humans occurs most frequently in rural areas where pastoralism is a major occupation, with higher endemicity in transhumant, semi-nomadic or fully nomadic societies (Craig et al., 1996; Macpherson, 2005). Dogs are always kept in such communities being used commonly for guarding, herding, hunting and/or companionship (e.g. van Kesteren et al., 2013). Individual families/households may own several dogs, and some large sheep ranches more than 20 dogs. A history of dog ownership has been identified as a risk factor for human CE (Campos-Bueno et al., 2000). Average worm burden is probably 50–200 but the intensity of infection is highly overdispersed with a few dogs having burdens >1000 worms. Although a variety of livestock species (including goats, cattle, yak, camels, horses and pigs) may be susceptible to CE, in endemic regions sheep are the most important intermediate host for *E. granulosus* (s.s.).

An increase in human CE cases admitted to hospital for surgical treatment is often an indicator of disease emergence, for example in northern Israel after border changes (Nahmias et al., 1992), in Kazakhstan after husbandry changes following independence from the Soviet Union in the early 1990s (Torgerson et al., 2003a), or in Cyprus after cessation of control measures in one region of the island (Economides et al., 1998). However increased case detection from retrospective surveys of hospital records or as a result of active mass screening programmes may also indicate 'emergence' though in fact is probably due to a lack of previous data (Craig et al., 2003). Following an increase in reported human CE cases, abattoir data should be scrutinised to establish ovine CE prevalence, and proactive surveys of owned and/or stray dog populations implemented to determine canine echinococcosis prevalence (and possibly identify risk factors) (Schantz, 1997; Gemmell et al., 2001a).

2.3. Prevention and control of cystic echinococcosis

Control of cystic echinococcosis and reduction or elimination of human CE as a public health problem has been successfully achieved, following long intervention periods (>10–20 years) from the 1960s, in several regions where the dog–sheep transmission cycle predominates: notably in New Zealand, Tasmania, Cyprus, Uruguay and Argentina (Rio Negro) (Craig and Larrieu, 2006). The world's largest hydatid control programme has been underway in western China since 2007 (WHO, 2011). For epidemiological studies and surveillance of control programmes, diagnostic tests and approaches in animal hosts are vital and have traditionally relied on post-mortem parasitological findings in sheep (and other livestock) and on purgation of dogs (Craig, 1997). Advances in diagnosis of definitive hosts and livestock have however not progressed equally

over the last 20 years. Laboratory-based diagnostics (coproELISA, coproPCR) for canine echinococcosis have made a huge impact in epidemiological studies and in surveillance of control, however slaughter inspection of livestock, which has a limited sensitivity and specificity as described in Section 4.1 below, still remains the most commonly used method of diagnosis of CE in livestock (Lembo et al., 2013). The role of surveillance in control is discussed below.

3. Diagnosis and detection of *E. granulosus* (s.l.) in dogs

3.1. Necropsy and purgation to detect canine echinococcosis

Post-mortem examination (necropsy) of the entire small intestine (SI) for the presence of the small (3–7 mm) adult tapeworms is the gold standard for the detection of canine echinococcosis (Craig, 1997; Eckert et al., 2001). A number of methods based on necropsy are available for detection of echinococcosis in definitive hosts, including the 'sedimentation and counting technique' (SCT), the intestinal scraping technique, and the 'gut incubation in saline' technique (Deplazes and Eckert, 1996; Craig, 1997). The latter method is for recovery of worms in the field, wherein the SI should be opened and cut into 15 cm pieces then incubated for up to 1 h in saline at 37 °C; this enables the majority of living worms to drop off into the sediment for counting. The gut sections can then be scraped to remove any remaining attached/mucus-embedded worms for washing over a sieve into a black-backed tray (Craig, 1997). Biohazard safety measures should be in place throughout the process. Necropsy is 100% specific in areas not co-endemic for *Echinococcus multilocularis*. However in co-endemic regions care needs to be taken to differentiate adult worms of *E. granulosus* from *E. multilocularis* (2–3 mm); morphologically this is based on overall size, position of proglottid lateral pore (above mid-line in *E. multilocularis*, below mid-line in *E. granulosus*) and uterus shape (sacculated in *E. granulosus*) (Thompson and McManus, 2001). *Echinococcus* spp. tapeworms attach to the anterior SI, however *E. granulosus* s.l. are generally located in the anterior duodenum, while those of *E. multilocularis* preferentially attach more distally in the mid/posterior duodenum. In mixed infections this separation in the SI may be obvious—even with a space of several cm between the two species. Species confirmation can also be achieved by extracting DNA from adult worms recovered at necropsy from the SI (e.g. Sobrino et al., 2006). Sensitivity of necropsy for *E. granulosus* is high (>97%) but in very low worm burdens (<6 worms) there is a chance of false negative results, especially if SCT is not carried out. Where culling of unwanted dogs occurs or is possible, the use of necropsy can provide very useful information on worm presence, worm burden data and also provide panels of faecal samples from parasitologically defined animals for coprotest standardisation (Buishi et al., 2005a; Ziadinov et al., 2008). However, the accurate diagnosis of truly negative animals for test standardisation and evaluation may be challenging—especially in the presence of animals with low worm burdens.

Purgation using arecoline plant extracts (historically) or synthetic salts (arecoline hydrobromide) has been a pre-mortem gold standard for detection of canine echinococcosis for over 100 years. It was used in the Icelandic hydatid control programme in the late 1880s as well as for surveillance in several ultimately successful hydatid control schemes in the 1960–1990s (for example in New Zealand, Tasmania, Uruguay, and Chile) (Craig and Larrieu, 2006). Logistically, arecoline purgation (at 2 mg/kg in a gavage solution or as tablets) is difficult to implement for more than a few dogs: requiring trained man-power, owner compliance and biohazard containment in the field, followed by time-consuming processing of purge samples (achieved by field observation of fixed purge in 5–10% formalin or boiled sample), and lab-based microscopic examination (Craig et al., 1995) or molecular characterisation of worms (De la Rue et al., 2011). On an empty stomach most dogs will purge between 30 and 60 min. The very high specificity (99–100%) of purgation is the key advantage together with a potential result within 1–2 h, and additionally it can provide a useful educational role for dog owners (Gemmell, 1990). However the sensitivity of purgation may be low compared to necropsy especially in low intensity infections or when full purge does not occur (Gemmell, 1973; Craig et al., 1995; Lahmar et al., 2007b). Furthermore some dogs fail to purge at all; weak, pregnant, young and old animals are often not treated; and owners may refuse permission for valued animals due to the potential risk of toxicity (cardiovascular collapse—sometimes treatable with atropine) or gut penetration. Various studies indicate a range of sensitivities for arecoline purgation from <40–75% with increased sensitivity after a second purge (Lahmar et al., 2007b). Despite these drawbacks purge data has been successfully used to estimate worm burdens to help determine transmission dynamics especially for *E. granulosus* infection of owned dogs, when of course necropsy is not possible (Budke et al., 2005b; Ziadinov et al., 2008; Hartnack et al., 2013).

3.2. Serology for canine echinococcosis

Serodiagnostic tests for canine echinococcosis were considered as a serious potential route for practical testing of dogs for *E. granulosus* infection and initially, as a potential substitute for arecoline purgation. In the 1980s, research primarily at Melbourne University investigated the use of native (or recombinant) antigen extracts from adult, protoscolex or oncosphere stages for detection of serum antibodies to *E. granulosus* (Jenkins and Rickard, 1986; Gasser et al., 1988). Specific IgG antibodies were detected by 2 weeks post-infection (wpi) in experimentally infected dogs though no correlation with worm burden was observed. Diagnostic specificity was good (>90%) but sensitivity generally poor (35–40%) with natural infections, and was much lower when compared directly to coproantigen detection (Gasser et al., 1988; Jenkins et al., 1990; Craig et al., 1995; Sakai et al., 1995). Further research to assess existing or develop better recombinant antigens may improve the sensitivity of serologic tests for canine echinococcosis (Carmena et al., 2006; Zhang and McManus, 2006) but currently coproantigen and coproPCR tests offer a much better diagnostic approach (Zhang and McManus, 2006).

3.3. Coproantigen ELISA for detection of canine echinococcosis

A specific and sensitive laboratory test for antigen detection in canid faecal samples (coproantigen) was considered to have the potential to replace arecoline purgation and to have the advantage over serology for detection of current infection (Babos and Nemeth, 1962; Allan et al., 1992; Deplazes et al., 1992). Coproantigen ELISA or coproELISA provides an alternative method for diagnosing canine echinococcosis, and both polyclonal and monoclonal antibodies have been used: directed against either somatic or excretory/secretory (ES) antigens. To create polyclonal antibodies against *Echinococcus* spp., rabbits were hyperimmunised with *Echinococcus* antigens, such as adult or protoscolex ES extracts (e.g. Benito and Carmena, 2005), or somatic extracts of adult tapeworms (e.g. Allan et al., 1992). Alternatively, monoclonal antibodies have been produced using donor mice hyperimmunised with *E. granulosus* somatic or ES antigens (e.g. Morel et al., 2013) (Table 1).

CoproELISAs are usually genus-specific for *Echinococcus* spp. (Allan and Craig, 2006), although depending on the endemic region and study aims, coproELISAs have been developed and validated to test for infection with *E. multilocularis* in foxes and dogs (e.g. Machnicka et al., 2003) or primarily for *E. granulosus* (Buishi et al., 2005a). For canine echinococcosis due to *E. granulosus* most authors

Table 1
Coproantigen ELISAs for diagnosis of *Echinococcus granulosus* s.l. in dogs.

CoproELISA	*n* (exp, pm, purge)	Sensitivity (%)	Specificity (%)	Cross reactions[a]	Reference
R anti EgW	410 (exp, pm, purge)	83	96	Th	Allan et al. (1992), Craig et al. (1995)and Buishi et al. (2005)
R anti EgWES	155 (exp, pm)	87	98	Th	Deplazes et al. (1992)
Mab EgWES, EmA9	13 (exp)	100	96	Th, Tm	Malgor et al. (1997) and Nonaka et al. (2011)
R anti EgPxES	200 (pm)	78.4	93.3	?	Benito and Carmena (2005)
Mab EgES, EgC1/EgC3	16 (exp)	100	100	Th	Casaravilla et al. (2005)
R anti EgWWES, S anti EgWFT	55 (exp, pm)	92	80	*Taenia*	Huang et al. (2008)
R anti EgW	411 (exp, purge)	92	86.5	*Taenia*	Pierangeli et al. (2010)
Mab Eg9ES	24 (exp, pm)	86.5	86.4	*Taenia*	Morel et al. (2013)
R anti EgW[b]	35 (exp, pm)	60	93	*Taenia*	Commercial kit[b] Huang et al. (2014)

R = rabbit antibodies; S = sheep antibodies; Mab = monoclonal antibodies; W = adult somatic; WES = adult excretory/secretory; Px = protoscolex; WFT = adult freeze-thaw extract; exp = experimental infection with necropsy; pm = post mortem of natural infected dogs; purge = arecoline purge examination; Eg = *E. granulosus*; Em = *E. multilocularis*; Th = *Taenia hydatigena*; Tm = *T. multiceps*;

[a] Excludes *Echinococcus multilocularis*.

[b] From Xinjiang Tiankang Animal Husbandry Biotech Co., Ltd., Urumqi, China.

report reasonable sensitivity (78–100%) (Allan et al., 1992; Benito and Carmena, 2005; Buishi et al., 2005a) and good genus specificity from 85% to greater than 95% (Allan et al., 1992; Benito and Carmena, 2005; Buishi et al., 2005a), as well as a degree of prepatent detection (Deplazes et al., 1992; Jenkins et al., 2000). Where cross-reactions occur these generally appear to be caused by infection with *Taenia hydatigena*, the most common taeniid of dogs, and attempts to improve specificity by using monoclonal antibodies in coproELISAs have not been able to eliminate this problem (Malgor et al., 1997; Morel et al., 2013) (Table 1). CoproELISA sensitivity broadly correlates with worm burden of *E. granulosus* (Malgor et al., 1997; Fraser et al., 2002; Buishi et al., 2005a), however some low intensity infections (worm burdens <50–100) may give false negatives in coproELISA (Allan and Craig, 2006).

CoproELISAs offer several logistical advantages over purgation: not least due to the fact that faecal samples can be collected from the ground by one person, thus avoiding difficulties associated with restraining and purging dogs by multiple trained personnel (as well as the reduced biohazard risk associated with the process). Coproantigens are rich in carbohydrate/glycoprotein and thus generally very stable, can be detected in ground faecal samples after days of environmental exposure, and can be preserved in a 5–10% formalin solution for several months without refrigeration (see Allan and Craig, 2006). This is a great advantage for field-based studies, especially as echinococcosis often affects rural and relatively remote communities (Craig et al., 2007). Furthermore, ELISAs have the advantage that the reaction can be read visually without the need for expensive apparatus, and the labelled reagents used are stable and are easily stored for long periods of time without loss of activity. CoproELISA protocols are usually relatively straightforward and multiwell microtitre plates are easy to handle and wash, thus allowing for relatively large numbers of samples to be processed relatively quickly (Adkinson et al., 1988).

The commercial availability of coproELISAs for *E. granulosus* canine echinococcosis has been problematic, with two kits discontinued in Europe (Chekit Bommeli, Switzerland; Genzyme Virotech GmbH, Germany). Currently commercial tests appear restricted to three coproELISA kits for canine echinococcosis produced in China (i.e. Shenzhen Combined Biotech Co., Ltd.; Zuhai Special Economic Zone Haitai Biological Pharmaceuticals Co., Ltd.; Xinjiang Tiankang Animal Husbandry Biotech Co., Ltd.). These three China-based kits were recently assessed, against a parasitologically defined panel of dog faecal samples by the Institute of Parasitic Disease Prevention and Control, Sichuan CDC (also in China) and found to be of variable sensitivity and specificity, with the best kit providing a reported 60% sensitivity and 93% specificity (Huang et al., 2013).

Rational approaches to develop better coproELISAs might ideally be based on monoclonal antibodies raised against highly specific exposed surface or ES antigens of adult *E. granulosus* that are known also to occur in faecal samples of infected dogs. Early studies on taeniasis indicated that coproantigens were large molecular weight (>100 kDa) carbohydrates, as they were heat, formalin and protease resistant but sensitive to periodate treatment (Allan et al., 1992; Kohno et al., 1995). Direct biochemical analysis and fractionation of adult tapeworms and of positive coproantigen faecal supernatants from *E. granulosus* infected dogs, indicated antigens were indeed highly glycosylated containing β-galactose, *N*-acetyl-β-glucosamine, *N*-acetyl-D-glucosamine and sialic acid residues and probably derived from the surface glycocalyx of the adult tegument (Elayoubi et al., 2003; Elayoubi and Craig, 2004; Casaravilla et al., 2005). N- and novel O-linked glycans were confirmed in *E. multilocularis* coproantigens using mass spectroscopy, HPLC and enzymic sequencing (Hulsmeier et al., 2010). These latter authors used immunoaffinity purified glycans prepared using MabEmA9 antibody, previously used in some coproantigen tests for *E. multilocularis* or *E. granulosus* (Kohno et al., 1995; Malgor et al., 1997).

The purification of a specific set of *Echinococcus* glycoconjugates from the tegumental surface or ES products of adult tapeworms and subsequent production of species/genus specific monoclonal antibodies could be a better approach for development of coproantigen diagnostic antibodies for use in coproELISA. This could be further optimised if putative diagnostic monoclonal antibodies were also pre-screened/selected against similar purified extracts from *T. hydatigena* adult worms in order to avoid cross-reactive moieties. This latter differential screening approach was recently reported by Morel et al. (2013) in their attempts to produce a genus specific *Echinococcus* monoclonal antibody against crude adult ES, however despite the coproELISA being very good overall, cross-reactions still occurred with some *T. hydatigena* infected dogs. Use of surface glycoproteins/glycans might be more productive than immunising with crude ES antigens for production of monoclonal or polyclonal antibodies. Furthermore, use of a hybrid assay approach i.e. polyclonal antibody as capture (for maximum sensitivity) and a monoclonal as detection antibody (for maximising specificity) could provide a more robust sensitive and specific coproELISA test.

3.4. CoproPCR for detection of canine echinococcosis caused by E. granulosus s.l.

While coproantigen ELISAs provide a better overall and practical alternative compared to arecoline purgation for pre-mortem detection of canine echinococcosis, their lack of species specificity is a disadvantage especially for epidemiological studies. The amplification of small fragments of species-specific *Echinococcus* DNA in eggs or in faeces by polymerase chain reaction (PCR) was first reported

for *E. multilocularis* infections in foxes (Bretagne et al., 1993), with reduced inhibition and sensitivity subsequently increased by egg concentration through sieving and zinc chloride flotation of faecal samples (Mathis et al., 1996). Cabrera et al. (2002) applied this approach targeted to the mitochondrial cytochrome c oxidase subunit 1 (cox1) gene of *E. granulosus* as proof of principle for PCR identification of eggs of *E. granulosus* (with an analytic sensitivity of 4 eggs) isolated from adult tapeworms and faecal samples from necropsied dogs in Argentina.

In the first comprehensive development of a coproPCR test designed for species specific detection of *E. granulosus* G1 infection of dogs, Abbasi et al. (2003) amplified a new repeat sequence (EgG1*Hae*III) with an analytic sensitivity of 1 egg, and demonstrated 100% diagnostic sensitivity using total DNA extraction from faecal sediments of 34 naturally infected dogs from Jordan or Kenya. These authors also reported 100% specificity when applied to the DNA of *E. multilocularis* and various *Taenia* spp. (Abbasi et al., 2003). Further assessment of the 'Abbasi' coproPCR found that it was highly specific for *E. granulosus* sensu lato but could not reliably differentiate the genotypes of *E. granulosus* (i.e. *E. granulosus* s.s., *E. equinus*, *E. ortleppi* and *E. canadensis*) (Boufana et al., 2008). A coproPCR specific for *E. granulosus* s.s. (G1 genotype), was subsequently reported that used primers for the 12S rRNA mitochondrial gene to amplify DNA from taeniid eggs isolated by zinc chloride flotation from faeces of naturally infected dogs purged in Kazakhstan (Stefanic et al., 2004). These authors were also the first to differentiate natural mixed infections of *E. granulosus* and *E. multilocularis* using two PCRs on the same faecal samples, also all dogs with sole *Taenia* spp. infections tested negative to the *E. granulosus* PCR. This PCR was also successfully applied to specifically identify *E. granulosus* eggs in taeniid egg contaminated soil samples collected around homesteads in an endemic area of southern Kazakhstan (Shaikenov et al., 2004). Later developments for use of PCR with canid faeces included multiplex PCR for differentiation and species/strain specific identification of taeniid eggs after sequencing of PCR products including *E. granulosus* s.s. (G1–G3) and *E. canadensis* (G6/G7) (Dinkel et al., 2004; Trachsel et al., 2007). Further refinement for genotyping of the *E. granulosus* complex for molecular epidemiological studies, also used multiplex PCR to identify unequivocally tapeworm tissue at genus level (*Echinococcus*), species complex level (*E. granulosus* s.l.) and genotype level (*E. granulosus* G1–G10), but the PCR was not very sensitive (<40%) for detection of eggs purified from faecal samples (Boubaker et al., 2013) (Table 2).

Application of coproPCR to faecal samples from dogs experimentally infected with *E. granulosus* in the pre-patent period using the repetitive element of Abbasi et al. (2003) indicated unequivocal pre-patent detection of parasite DNA in faeces between 21 and 25 days post-infection (dpi) i.e. before egg production in faeces (Naidich et al., 2006; Lahmar et al., 2007b). In a direct comparison of arecoline purgation, coproELISA and coproPCR in pre-patent infections (21–33 dpi), Lahmar et al., (2007b) showed that coproELISA had a sensitivity of 82.8% compared to 25.9% for coproPCR, while both coproantigen and PCR positivity increased significantly with worm burden. Arecoline purgation for pre-patent infections gave a sensitivity of 43% after a single purge (Lahmar et al., 2007b).

The ability to perform PCR with faecal samples or extracts directly without first isolating taeniid eggs is an advantage especially when relatively large numbers of samples require testing. Usually total DNA is extracted from canid faecal samples (1–2 gm) using the commercial QIAamp DNA Mini Stool Kit (Qiagen, Germany), with one kit being able to process 50 samples. This approach has been used with at least two coproPCRs based on the EgG1 *Hae* III repeat (Abbasi et al., 2003) and the NADH dehydrogenase subunit 1 gene (ND1) (Boufana et al., 2013). The reliance on relatively expensive PCR thermal cycler machines is problematic in resource-poor endemic regions, and thus assessment of DNA amplification in normal water bath conditions has been evaluated with loop-mediated isothermal amplification (LAMP) which gave high specificity when parasite tissue or egg-spiked faecal samples were assessed for *E. granulosus* s.l. and *E. granulosus* s.s. (Salant et al., 2012; Ni et al., 2014), and also for *E. equinus*, *E. ortleppi*, *E. canadensis* and *E. felidis* (Wasserman et al., 2014).

Real-time PCR has been suggested to offer a number of advantages over conventional PCR for the detection of parasitic infections, including increased sensitivity and specificity, reduced reaction time and a quantitative estimate of the amount of DNA in the sample (which may relate to both the infectiousness of the sample and the possible burden of infection) (Bell and Ranford-Cartwright, 2002; Bretagne, 2003). A number of studies have investigated the use real-time PCR to detect *E. multilocularis*, with promising results (Dinkel et al., 2011; Knapp et al., 2014; Øines et al., 2014), and similar methods could be used for other species of *Echinococcus*.

Currently there are several PCRs published for the *E. granulosus* complex (Table 2) and their great value is in provision of absolute or extremely high specificity to the extent that a result can be taken as proxy to replace the finding of worms at necropsy or purgation. The latter is important because it has become increasingly difficult to undertake necropsy with unwanted dogs or to carry out arecoline purgation in owned animals. However, diagnosis based solely on PCR techniques is considered an unsuitable strategy for large-scale surveillance and screening programmes, due to the high labour intensity and high expense of the procedure. The most practical and cost-effective way to undertake testing of dogs on a large scale is to adopt a serial testing strategy based on primary screening of all samples using the coproELISA test, followed by testing of all positives using coproPCR (Mathis et al., 1996; Craig et al., 2003). This can be particularly beneficial in cases where the prevalence of infection (and therefore the positive predictive value of the ELISA test alone) is low. However, it may also be prudent to PCR test a random number (e.g. 20%) of coproELISA negatives because the correlation between coproantigen positive and coproPCR positive dogs is not always very clear due to low worm burdens, low egg counts, pre-patent infections and possibly coprophagia.

4. Diagnosis and detection of cystic echinococcosis in livestock

4.1. Slaughter and meat inspection for detection of CE in livestock

E. granulosus infection of livestock (CE) is most commonly diagnosed at necropsy—which in the case of sheep, goats, cattle and pigs will usually be during meat inspection (either in an abattoir or prior to consumption/sale), using visual inspection, palpation and/or incision (OIE, 2008). CE in horses (which is less commonly seen than in other livestock, caused by *E. equinus*) may be detected incidentally during routine necropsy as well as during meat inspection (Binhazim et al., 1992; Varcasia et al., 2008). Fully developed *E. granulosus* metacestodes in the intermediate host are generally identifiable as unilocular, fluid-filled cysts located in the viscera—most commonly, the liver and/or the lungs, although other organs and tissues may be involved, especially when burdens are higher (Liu et al., 1993; Thompson, 1995; Eckert et al., 2001).

Considering that each hydatid cyst develops from a single oncosphere, which is less than 40 μm in diameter when infective, it can take some time after infection for metacestodes to become visible to the naked eye. Although the rate of development of cysts is variable, it has been estimated that the rate of growth is between around 1 and 5 cm per year (Heath, 1973; Thompson, 1995). Early lesions of *E. granulosus* will generally appear as small white nodules, and can be easily missed (Liu et al., 1993)—making the sensitivity of diagnosis based on meat inspection quite low in early infections (for exam-

Table 2
CoproPCRs for diagnosis or detection of *E. granulosus* s.l. in dogs.

Gene	Species/genotype	Copro sample	Tissue	Specificity	Reference
cox1	*E. granulosus*	eggs	yes	High	Cabrera et al. (2002)
EgG1*Hae*III	*E. granulosus* s.l.	faeces	yes	Very high	Abassi et al. (2003)
12SrRNA	*E. granulosus* G1	eggs	yes	High	Stefanic et al. (2004)
cox1,NAD1	*E. granulosus* G1, G5, G6, G7	no	yes	Very high	Dinkel et al. (2004)
cox1 EgG1*Hae*III	*E. granulosus* G1-G7	faeces		High	Naidich et al. (2006)
cox1,NAD1, rrnS	*E. granulosus* Taenia spp	eggs	yes	High after sequencing	Trachsel et al. (2007)
NAD1	*E. granulosus* G1 *E. shiquicus*	faeces	yes	High	Boufana et al. (2013)
Mitoch., Nuclear	*E. granulosus* G1-G10	eggs	yes	High	Boubaker et al. (2013)
LAMP	*E. granulosus* G1	eggs	yes	High	Salant et al. (2012)
LAMP	*E. granulosus* G1 E. canadensis	no	yes	High	Wasserman et al. (2014)
LAMP	*E. granulosus* G1	faeces	yes	Good	Ni et al. (2014)

ple, in young animals). The sensitivity of necropsy diagnosis of CE can be increased by thinly slicing liver and lung tissue for examination (Lloyd et al., 1991, 1998), The specificity of meat inspection may also be imperfect due to other infections or conditions resulting in similar lesions—in particular, the metacestode stage of *T. hydatigena*, which can also present as cystic lesions (*Cysticercus tenuicollis*). Other differential diagnoses of echinococcosis include other parasitic infections (such as *Toxocara* spp., *Ascaris suum*, *Fasciola* spp. and *Dictyocaulus filaris*), granulomas, calcified tuberculosis lesions, caseous lymphadenitis (*Corynebacterium pseudotuberculosis*), and congenital cysts (Eckert et al., 2001; Gemmell et al., 2001a). In areas coendemic for both *E. granulosus* s.l. and *E. multilocularis*, cysts of *E. multilocularis* in pigs (which are susceptible to both species) may be confused for CE. The specificity can be improved by histopathological, immunohistochemical or PCR analysis of suspect lesions (Eckert et al., 2001; Zhang and McManus, 2006). Several studies involving the DNA typing (species and genotypes) of cysts discovered at slaughter inspection have proved the usefulness of this approach (Bardonnet et al., 2003; Casulli et al., 2008; Boufana et al., 2014), and real time PCR has also be used to detect and distinguish *E. granulosus* genotypes using cyst material (Maurelli et al., 2009; Pestechian et al., 2014).

Appropriate action should be taken if CE lesions in livestock are identified or suspected during necropsy or meat inspection. From a surveillance perspective, the cyst viability/fertility, cyst location in the body (for example, the liver:lung ratio), age of the animal, and origin of the animal should be recorded (Eckert et al., 1982; Gemmell et al., 2001a); whereas from a disease control perspective, it is important that affected tissues are condemned and disposed of appropriately. Tissues should ideally be placed in sealed containers and incinerated or rendered, as burying, use of garbage tips, or feeding directly to dogs are all likely to perpetuate transmission.

4.2. Serological diagnosis of CE in livestock

The G1 genotype of *E. granulosus* s.s. (sheep strain) is globally the dominant species of *Echinococcus* and responsible for the majority of zoonotic CE cases. Serological diagnosis of ovine echinococcosis has therefore long been considered a potentially important tool for epidemiological studies in endemic areas, as well as for possible surveillance of hydatid control programmes (Lightowlers, 1990; Craig, 1997). It has been known for many years that sheep infected experimentally with *E. granulosus* can mount detectable specific IgG responses within weeks (Sweatman et al., 1963; Blundell-Hasell, 1969; Yong and Heath, 1984) and that specificity was reasonable against panels of taeniid antigen preparations (Craig et al., 1981). However serum antibody levels varied greatly in natural CE infections resulting in reduced sensitivity and cross-reactions with *T. hydatigena* or *Taenia ovis* infected animals (Yong and Heath, 1984; Ming, 1986). Attempts to increase sensitivity and specificity by biochemical or immunochemical purification of native antigens (mostly hydatid fluid origin, e.g. antigen B) or recombinant AgB, resulted only in modest improvements that at best allowed for overall discrimination on a flock, herd or group basis (Lightowlers et al., 1984; Ris et al., 1987; Ibrahem et al., 1996).

Application of techniques from human CE diagnostics, such as immunoblot, to diagnosis of ovine CE have been reported to have high specificity (Moro et al., 1997; Gatti et al., 2007) and/or sensitivity (Dueger et al., 2003). However, in natural CE infections in sheep, antibody levels were variable—especially with increased host age and cyst numbers. One of the more comprehensive evaluations of ELISA for serodiagnosis of ovine CE assessed antibody responses against native antigen B from hydatid cyst fluid, a crude *E. granulosus* protoscolex extract, and recombinant EG95 oncosphere antigen, using panels of sera taken from experimentally infected or abattoir inspected sheep (Kittelberger et al., 2002). The best combination of sensitivity (62.7%) and specificity (95.8%) was obtained by these authors with the protoscolex extract as diagnostic antigen (Table 3). The detection of circulating fragments of *Echinococcus* DNA, if possible, could result in absolute specificity with higher sensitivity for laboratory-based diagnosis of CE in livestock (McManus, 2014).

One practical problem for all studies that use serum panels from natural CE infections in livestock is that unless full necropsy and total organ (liver/lungs) slicing is carried out in conjunction with histology (or PCR) for small lesions (e.g. Gatti et al., 2007), it is very difficult to confidently identify an animal as hydatid-free, or conversely non-infected with other larval taeniid species, and thus both sensitivity and specificity could be based on equivocal data.

4.3. Ultrasound scanning for CE in sheep and goats

In humans, ultrasound (US) scanning for hepatic CE has been the mainstay of both clinical investigations and especially for mass screening in endemic communities (Macpherson et al., 2003), after its potential was first recognised in the 1980s (Gharbi et al., 1981). Use of imaging techniques for CE detection in sheep initially reported radiography to identify pulmonary infections (Wyn-Jones and Clarkson, 1984), and subsequently the potential application of US to screen for ovine CE was suggested (Craig, 1993), with

Table 3
Serodiagnostic tests for antibody detection in ovine cystic echinococcosis.

Antigen	Test	*n* (exp., nat)	Sensitivity (%)	Specificity (%)	Reference
HCF	IHA	99 (nat.)	25–50	40–60 (Th)	Blundell-Hasell (1969)
HCF	Arc5-IEP	42 (exp. >1 yr)	23.8	61–89 (Th,To)	Yong and Heath (1979)
PxES	ELISA	41 (exp. 1yr)	85.7	59.3 (Th, To)	Ris et al. (1987)
nAgB	ELISA	59 (nat.)	90	99 (Th)	Ibrahem et al. (1996)
rAgB			25		
8,16,20 AgB subunits	EITBlot	137 (nat.)	73	98.6 (controls)	Moro et al. (1997)
8,16,20 AgB subunits	EITBlot	199 (nat.)	91.4	38.3 (no lesions)	Dueger et al. (2003)
nAgB	ELISA	23 (exp.), 226CE, 1069	11.2	96.7	Kittelberger et al. (2002)
nPx		(nat.)	62.7	95.8	
rEG95			5.2	95.8	
HCF	ELISA	247 (nat.)	89.2	89.5	Gatti et al. (2007)
nAgB			86.4	92.8	

HCF = crude hydatid cyst fluid; PxES = protoscolex ES antigen; nAgB = native antigen B; rAgB = recombinant antigen B; 8, 16, 20 AgB = specific bands of antigen B after blot; nPx = somatic extract of protoscoleces; rEG95 = recombinant oncosphere antigen; IHA = indirect haemagglutination test; arc5- IEP = arc5 immunoelectrophoresis test; ELISA = enzyme-linked immunosorbent assay; EITBlot = immunoelectrotransfer blot; exp. = experimental infections with post mortem; nat. = natural CE infections identified at abattoir inspection; Th = cysts of *T. hydatigena*; To = cysts of *T. ovis*.

first reports in the late 1990s in the Turkana district of Kenya (Maxson et al., 1996; Sage et al., 1998; Njoroge et al., 2000) (Table 4). Both hepatic and lung cysts (right lobe) could be imaged in standing/wool-clipped sheep and goats, with a reported specificity of 82–98% when compared with necropsy findings (Maxson et al., 1996; Sage et al., 1998). False positive images were caused by the presence of cysts of *T. hydatigena* which appeared to be the main specificity problem (Table 4). Assessment of sensitivity of ultrasound for ovine CE has been more problematic because in some studies only image positive animals were subsequently slaughtered (Maxson et al., 1996; Lahmar et al., 2007a). In one study in Tunisia, US positive sheep showed cyst numbers of 3.8–4.8 cysts per animal at slaughter with US only detecting around one third of all hydatid cysts (Lahmar et al., 2007a). Interestingly in two studies of naturally infected sheep flocks in Tunisia (Lahmar et al., 2007a) and Sardinia (Dore et al., 2014), US could correctly identify the proportion of 'active cysts' (i.e. types CE 1 and 2) and 'transition/inactive' cysts (i.e. types CE 3–5) to be respectively 5–7% and 93–95%. Hydatid cyst size detectable by US in sheep ranged from 0.9–6.0 cm (Dore et al., 2014).

The most two comprehensive assessments of US were in goats (primarily) in NW Kenya (Sage et al., 1998) and in sheep in Sardinia (Dore et al., 2014), when all scanned animals were subject to slaughter inspection. The more recent study in Sardinian sheep benefited from the availability of a modern high resolution microconvex transducer and demonstrated a reasonably high sensitivity (88.7%) (Dore et al., 2014). However, the specificity of US was lower (75.9%) in the Sardinian study compared to the Kenyan assessment (97.6%) by Sage et al. (1998). Both these studies indicate that mass US scanning for CE in small ruminants is cost-effective, practical and can provide sensitivity and specificity equal to or better than current serology.

5. Diagnosis and detection of echinococcosis in wildlife

5.1. E. granulosus *s.l. in wild carnivores*

Cystic echinococcosis is the most widespread form of human echinococcosis, and is most commonly caused by the G1 genotype of *E. granulosus* (88% of human cases), with approximately 11% of human infections attributed to *E. canadensis* (Alvares Rojas et al., 2014). Domestic dogs are final hosts for both *E. granulosus* s.s. (Eckert and Deplazes, 2004) and *E. canadensis* (e.g. Bart et al., 2006). Furthermore, domestic dogs pose the largest risk of human infection due to their close association with humans (Budke et al., 2005b). As such, studies on echinococcosis often focus on domestic dogs. However, many wild canids also function as final hosts for *E. granulosus* and *E. canadensis*, and may pose risks to humans, or act as wildlife reservoirs for these parasites (Jenkins, 2006; Barnes et al., 2012). The techniques used to assess *Echinococcus* spp. infection status in dogs such as necropsy, microscopic analysis of collected faecal samples, coproELISA and coproPCR, or combinations of these, can also be applied to wild canids.

Necropsy and examination of the intestines of road killed, hunted or culled animals can help identify wild canid host species that are infected with *Echinococcus* spp. For example, examination of carcasses of golden jackals (*Canis aureus*), red foxes (*Vulpes vulpes*) and grey wolves (*C. lupus*) in Iran found *E. granulosus* s.l. prevalences ranging between 0 and 100% (Dalimi et al., 2002; Dalimi et al., 2006; Beiromvand et al., 2011). Necropsy was also

Table 4
Ultrasound for detection of cystic echinococcosis in sheep and goats.

Animals	Region	*N* (PM)	Sensitivity (%)	Specificity (%)	False positive	Reference
Sheep and goats	NW Kenya	28 (+ only)	nd	82.1	Th cysts	Maxson et al. (1996)
Sheep and goats	NW Kenya S. Sudan	300 (all)	54.4	97.6	Th cysts	Sage et al. (1998)
Sheep	Argentina	22 (all)	100	100	none	Guarnera et al. (2001)
Sheep	Tunisia	18 (+ only)	nd	100	none	Lahmar et al. (2007)
Sheep	Sardinia	120 (all)	88.7	75.9	Th, Dicrocoelium	Dore et al. (2014)

PM = post mortem; (+ only) = image positives slaughtered only; (all) = all animals scanned were slaughtered; nd = not done; Th = *T. hydatigena* cysts; *Dicrocoelium* = livers with heavy *D. dentriticum* infection.

Table 5
Detection of *E.granulosus* s.l. in wild canids.

Canid species	*Echinococcus* spp.	Method	Reference
Grey wolf (*Canis lupus*)	*E. granulosus* s.l.	Necropsy/microscopy	Rausch and Williamson (1959), Foreyt et al. (2008), Abdbekova and Torgerson (2012), Arbabi and Hooyshar (2006), Bagrade et al. (2009), Hirvela-Koski et al. (2003), Shimalov and Shimalov (2000)
	E. granulosus s.s.	Necropsy/PCR	Sobrino et al. (2006), Breyer et al. (2004)
	E. canadensis	Scats/necropsy/PCR	Moks et al. (2010), Bryan et al. (2012), Guerra et al. (2013)
Coyote (*C. latrans*)	*E. granulosus* s.l.	Necropsy/microscopy	Liu (1970)
Dingo (*C. familiarisdingo)*	*E. granulosus* s.l.	Necropsy/microscopy	Jenkins and Morris (2003), Browns and Copeman (2003), Jenkins et al. (2008)
Golden jackal (*C. aureus*)	*E. granulosus* s.l.	Necropsy/microscopy	Dalimi et al. (2002, 2006), Arbabi and Hooyshar (2006)
	E. granulosus G1	Scats/PCR	Lahmar et al. (2009), Beiromvand et al. (2011)
Black-backed jackal, (*C. mesomelas*)	*E. granulosus* s.l.	Necropsy/microscopy	Nelson and Rausch (1963)
African wild dog (*Lycaon pictus*)	*E. granulosus* s.l.	Necropsy/microscopy	Nelson and Rausch (1963)
Red fox (*Vulpes vulpes*)	*E. granulosus* s.l.	Necropsy/microscopy	Jenkins and Craig (1992), Dalimi et al. (2002), Arbabi and Hooyshar (2006)
		Scats/PCR	Lahmar et al. (2009), Beiromvand et al. (2011)
Zorro fox (*Dusicyon culpaeus*)	*E. granulosus* s.l.	Necropsy/microscopy coproELISA	Schantz and Lord (1972) Acosta-Jamett et al. (2014)
Grey fox (*Pseudalopex griseus*)	*E. granulosus* s.l.	Necropsy/microscopy	Zanini et al. (2006)
Ethiopian wolf (*C. simensis*)	*E. granulosus* s.l.	Scats/PCR	van Kesteren et al. (2015)

used to identify African wild canids as hosts for *E. granulosus* s.l., including black-backed jackals (*C. mesomelas*) and African wild dogs (*Lycaon pictus*) (Nelson and Rausch, 1963). In Australia necropsy and examination of intestines has identified dingoes (*Canis familiaris dingo*) (Brown and Copeman, 2003; Jenkins and Morris, 2003; Jenkins et al., 2008) and red foxes (Jenkins and Craig, 1992) as wild canid hosts for *E. granulosus* s.l.. In Argentina, 1/81 (1.2%) of necropsied grey foxes (*Pseudalopex griseus*), were found to have adult *E. granulosus* s.l. (Zanini et al., 2006). Necropsy and microscopic examination of intestines was used to identify 8 of 41 (19.5%) necropsied grey wolves infected with *E. granulosus* s.l. in Kazakhstan (Abdybekova and Torgerson, 2012); 1/34 (2.9%) of wolves in Latvia (Bagrade et al., 2009); 6/52 (11.5%) of wolves in Belarus (Shimalov and Shimalov, 2000); 60/200 (30%) of wolves in Alaska (Rausch and Williamson, 1959); 39/63 (61.9%) wolves in Idaho; and 38/60 (63.3%) of wolves in Montana (Foreyt et al., 2009). The same methodology was used to identify coyotes (*Canis latrans*), as final hosts for *E. granulosus* s.l. in California, with 7/173 (4%) found to be infected (Liu, 1970).

Necropsy and examination of tapeworms found in intestines can be combined with more modern molecular methods to more accurately identify species or genotypes of *Echinococcus* spp. present in wild canids. PCR amplification of DNA and gel separation (PCR-RFLP) of parasite tissues found one of 26 necropsied grey wolves from Estonia was infected with *E. canadensis* (Moks et al., 2006); and in Canada, Schurer et al. (2014) used PCR analysis on adult worms harvested from necropsied wolves to identify G8 and G10 genotypes of *E. canadensis*. *E. granulosus* s.s. (G1 genotype) was similarly confirmed in necropsied grey wolves in Spain (Sobrino et al., 2006) and Bulgaria (Breyer et al., 2004) (Table 5).

Pre-mortem methods have also been applied to studies on *Echinococcus* spp. in wild canids. In Tunisia, Lahmar et al., (2009) collected faecal samples (scats) from golden jackals and red foxes, and analysed these using microscopy, followed by faecal DNA extraction in order to confirm the presence of *E. granulosus* s.l.. Similar methods (microscope examination of samples followed by DNA extraction and PCR) were applied by Bryan et al. (2012), who identified strains G8 and G10 of *E. canadensis* from grey wolf faecal samples collected in Canada. In Portugal, grey wolf scats were tested by coproPCR and confirmed the presence of E. canadensis (G6/G7 genotype) (Guerra et al., 2013). Recently the collection of faecal samples followed by microscopy and PCR analysis was also used to confirm the rare Ethiopian wolf (*C. simensis*) as a final host for *E. granulosus* s.l. although its role in transmission is not clear (van Kesteren et al., 2015). Molecular diagnosis has now confirmed the presence of *E. granulosus* s.s. in wild lions and hyenas in East Africa (Kagendo et al., 2014).

5.2. Cystic echinococcosis in wild herbivores

The potential role of wild herbivores or ungulates as reservoir hosts of CE in the transmission of *E. granulosus* s.s. is an often asked question, and one which prior to the availability of molecular diagnostic tools has not been very easy to determine. It has been known for at least 50 years that CE cysts may occur in wild populations of a wide range of mammalian families including: cervids e.g. moose (*Alces alces*) and caribou (*Rangifer tarandus*); bovids e.g. buffalo (*Synceros caffer*), various antelope and gazelle species from Africa e.g. wildebeest (*Connochaetes taurinus*) or Asia e.g. Tibetan gazelle (*Pantholops hodgsoni*); camelids e.g. Llama (*Lama glama*), Alpaca (*Lama pacos*); equids e.g. zebra (*Equus quagga*); suids e.g. wild boar (*Sus scrofa*), warthog (*Phacochoeros aethiopicus*); hippopotamids (*Hippopotamus amphibius*); caprids/ovids e.g. blue sheep (*Pseudois nayaur*), mouflon (*Ovis gmelinii anatolica*); lagomorphs e.g. hare (*Lepus europaeus*); marsupials e.g. wallabies/kangaroos (*Macropus spp.*) and wombats (*Vombatus ursinus*) (Schantz and Lord, 1972; Macpherson and Craig, 1991; Bowles and McManus, 1993; McManus and Thompson, 2003; Rausch, 2003; Mwambete et al., 2004; Huttner et al., 2008; Huttner and Romig, 2009).

Molecular genotyping indicates a rather complex pattern of *Echinococcus* spp. that may cause CE in humans, livestock and wildlife in Sub-Saharan Africa (Huttner and Romig, 2009) and to an extent in North America and Eurasia (Alvares Rojas et al., 2014). The discovery of *E. felidis* in lions and its potential occurrence in a range of wild herbivores may explain several reports of wildlife CE in Sub-Saharan Africa, but further molecular diagnostic studies are required (Huttner and Romig, 2009). Zoonotic infections are dominated by *E. granulosus* s.s., which apart from livestock intermediate hosts, has to date only been molecularly confirmed in a few wildlife species, including kangaroos/wallabies, wild boar, warthog, Turkish mouflon, and wildebeest (Thompson and McManus, 2002; Huttner et al., 2008; Simsek and Eroksuz,

2009; Kagendo et al., 2014). In these latter wildlife intermediate hosts there is only real evidence for kangaroos/wallabies providing a significant wildlife reservoir via wild definitive hosts (dingoes, dingo-dog hybrids) in the transmission of *E. granulosus* s.s. and potential spillover into domestic transmission with associated zoonotic implications (Grainger and Jenkins, 1996; Jenkins et al., 2008).

5.3. Cystic echinococcosis in captive mammals

There have been numerous reports of natural infections of echinococcosis in captive mammals (zoos, safari parks, etc.), but these have been most frequently concerned with necropsy findings of hepatic lesions of *E. multilocularis* (alveolar echinococcosis) in primates, including great apes, macaques and lemurs (e.g. Rehmann et al., 2003; Bacciarini et al., 2004; Umhang et al., 2013). Diagnosis was usually based on classical post-mortem findings of cyst morphology and histology, but also specific serology and increasingly DNA analyses. Furthermore almost all cases in European zoos were associated with local transmission/contamination with eggs of *E. multilocularis*.

Cystic echinococcosis has also been diagnosed in captive wild mammals in Europe and Asia. However in some cases animals were likely infected prior to transfer to the zoo or collection. For example, a cluster of *E. granulosus* CE was confirmed by PCR and serology in pig-tailed macaques (*Macaca nemestrina*) in a primate colony at a German research institute after importation of animals from Slovenia, a known CE endemic country (Plesker et al., 2001). A zebra (*Equus burchelli antiquorum*) that suddenly died in Taipei zoo (12 years after import from South Africa) was found at post-mortem to be infected (liver and lungs) with 7 unilocular cysts (3–8 cm) that showed classic *E. granulosus* s.l. hydatid morphology and histology with presence of protoscoleces, however molecular confirmation of species/genotype was not carried out (Chiou et al., 2001). Similarly, CE infection in a Burchell's zebra born in UK has been described and confirmed by PCR and DNA analysis to be caused by *E. equinus*, and considered to be locally acquired probably through egg-contaminated feed (Boufana et al., 2012). These latter authors also molecularly confirmed CE caused by *E. granulosus* s.s. (G1) in a red-tailed guenon monkey (*Cercopithecus ascanius*) and also from post mortem recovered cysts removed from a Philippine spotted deer (*Rusa alfredi*), both held in UK zoos; a second deer (imported from France) was confirmed by molecular diagnosis to have CE cysts caused by *E. ortleppi* (G5). Interestingly, cysts recovered at necropsy from the abdominal cavity of a lemur (*Varecia rubra*) born in a UK zoo was confirmed by *cox*1 gene amplification to be *E. equinus* (G4): known to be endemic in UK, but never previously recorded in a primate (Boufana et al., 2012).

6. Data collection, analysis and interpretation for echinococcosis in animals

6.1. Approaches for surveillance

Surveillance is of central importance to the investigation of echinococcosis in humans and animals and is essential during any control scheme. Despite being a cornerstone of disease control, the concept of 'surveillance' is often misunderstood. It is defined by the World Organisation for Animal Health as "the systematic ongoing collection, collation, and analysis of information related to animal health and the timely dissemination of information so that action can be taken" (OIE, 2012). As such, surveillance is distinct from disease monitoring, which does not imply that any action will be taken as a result of the findings. Because surveillance is an active process, it is important to consider the aims of surveillance and the ability to respond and react to the results prior to any data collection. The three most common reasons for surveillance are to establish whether a disease control intervention is needed in a community (i.e. to establish the absolute and relative impacts of echinococcosis in the community in question); to formulate an appropriate intervention; and to evaluate any control schemes (through both initial collection of baseline data and ongoing data collection during the control scheme) (Schantz et al., 1995; Schantz, 1997).

As a wide number of different hosts may be involved in the *E. granulosus* lifecycle, a comprehensive surveillance strategy can become very involved. Minimal requirements for establishing a 'baseline' for ongoing surveillance have been suggested as follows (Eckert et al., 1982):

- Identify agents/agencies involved in the collection of data.
- Describe the intended methods of data interpretation and analysis.
- Establish the age-specific prevalence and geographic distribution of CE in intermediate hosts (including humans).
- Establish the prevalence of echinococcosis in definitive hosts.
- Establish human-associated risk factors for canine infection, and evaluate canine movements.
- Estimate the economic effects of echinococcosis from human health and animal productivity perspectives.

This provides a useful general framework for considering surveillance strategies. However, the methods of achieving these aims will vary considerably depending upon the particular situation at hand. For example, surveillance of echinococcosis in nomadic, semi-nomadic or transhumant situations will usually be very different from the approach adopted in pastoral areas of rich, developed countries with well-developed infrastructures. Additionally, there have been a number of important technological developments since these guidelines were first created, which should be considered when planning a surveillance strategy. These include developments in diagnostic testing such as coproantigen testing (Allan et al., 1992; Deplazes et al., 1992), human serology (Gottstein, 1992), PCR testing (McManus, 1990), and portable ultrasonography (Macpherson et al., 1987). Developments relating to the interpretation of data collected include spatial analysis (Mastin et al., 2011), Bayesian analysis (Torgerson et al., 2003b), and latent variable techniques for diagnostic test interpretation (Hartnack et al., 2013). Another recent advance is the economic evaluation of human illness using DALYs (Disability-Adjusted Life Years) (Murray, 1994; Budke et al., 2004).

Data sources are of central importance to any surveillance scheme. The terminology associated with this can be confusing, especially in the case of animal pathogens (Gibbens et al., 2003; Hoinville et al., 2013), but approaches to data collection are commonly described as either 'passive' or 'active'. Active surveillance adopts an active 'case finding' approach, and is generally conducted by investigators. Most surveys and censuses are included in this category. Passive surveillance is an ongoing process whereby observers routinely report or record the outcome of interest (Dufour and Hendrikx, 2009; Hoinville et al., 2013), such as ongoing abattoir surveillance during meat inspection. The two major 'data streams' of use for passive surveillance of cystic echinococcosis are abattoir surveillance for infection in livestock, and hospital surveillance for human infection (Schantz, 1997). Both of these forms of surveillance require a functioning recording and reporting system—ideally one which is managed centrally. Designation of echinococcosis as a reportable or notifiable disease can also assist passive surveillance. Whilst both abattoirs and hospitals can also be used for active surveillance (especially in countries where reporting systems and infrastructure do not permit effective passive surveillance), the primary methods of active surveillance for echinococcosis are ultrasound scanning surveys for human infec-

tion and surveys of infection in the definitive host (such as necropsy, purgation and coproantigen/coproPCR surveys). The use of sentinel animals for estimation of levels of pasture contamination (Gemmell and Johnstone, 1977; Eckert et al., 1982; Lloyd et al., 1991) is generally considered a form of active surveillance.

6.2. Surveillance of intermediate hosts

Abattoir surveillance of echinococcosis is useful for general assessment of overall transmission risk (possibly through the use of mathematical modelling, Roberts et al., 1987), as well as providing information on species of intermediate host of potential importance to the transmission cycle (Thompson and McManus, 2001). It is also of particular use for the monitoring of the efficacy of a control scheme (Gemmell, 1973), especially in the later stages, due to the high sensitivity of this method for detection of environmental contamination (Schantz, 1997), given consideration to the time lag between infection and cysts visible on gross examination.

In order for the recording and reporting system to function well, abattoir surveillance requires suitably trained staff and access to laboratories for confirmation of suspected cases (ideally using molecular epidemiologic methods to identify the species/genotype(s) involved; but failing that, histopathology). Effective passive surveillance of echinococcosis through meat inspection in abattoirs or slaughter slabs is relatively rarely found in developing countries, and most abattoir surveillance in these countries is based upon active surveillance (e.g. planned abattoir-based surveys). Another challenge associated with abattoir surveillance is that of selection bias—partly due to variations in abattoir use and partly due to spatial variations in abattoir catchment areas. In particular, the age structure of the animals slaughtered in the abattoir should be considered when interpreting any results, as the prevalence of infection in intermediate hosts is age-dependent. Failure to account for this if younger animals are predominantly tested will tend to under-represent the true infection pressure/risk (Torgerson and Heath, 2003; OIE, 2008). Additionally, given that abattoirs will generally deal with animals of particular ages (often very young animals bred for meat, or older animals which are no longer economically viable), additional planning may be required if a representative sample of animals of different ages is desired (Schantz, 1997).

The other main source of selection bias when dealing with abattoir data results from the difficulty in identifying a clear study population (in a geographical or animal management context). That is, the selection of a particular abattoir may not be based solely on location, and some people may choose not to use an abattoir at all. In order to address the first issue, efforts should always be made to identify the origin of animals arriving at the abattoir. Failure to do this could also lead to difficulties in the later stages of an echinococcosis control scheme, when case-finding becomes more important and identification of the origin of infected animals is required in order to increase surveillance or apply additional control measures. In the case of ongoing surveillance, the origin of all animals arriving at the abattoir would be advantageous, as this information will be needed to identify areas of high endemicity. However, this would generally require some form of animal and farm identification system, which may not be logistically or financially feasible in developing countries, and is not always effective in many developed regions. The second issue is that abattoirs may not be used by certain communities. Home slaughter is known to be an important risk factor for echinococcosis in dogs (Buishi et al., 2005a; Acosta-Jamett et al., 2010a), and is commonly practiced by subsistence farmers and within nomadic or seminomadic communities, which are also more likely to be affected by echinococcosis (Zinsstag et al., 2013). Surveillance of infection in intermediate hosts based solely on abattoir surveillance will tend to under-represent or exclude animals from these communities, and efforts may therefore need to be made to address this.

An important consideration when interpreting the results of surveillance in intermediate hosts is the time lag usually observed between infection and detectable signs. The only data sources which can give an accurate reflection of the current risk of infection are those based on infection in the definitive host or on environmental contamination. Although methods based upon egg extraction from soil samples have been applied for the direct estimation of levels of environmental contamination (Craig et al., 1988; Shaikenov et al., 2004; Matsuo and Kamiya, 2005), these can be quite labour intensive—especially when considering large areas of possible contamination. Another approach to investigation of environmental contamination with *Echinococcus* spp. eggs is to use sentinel animals (Gemmell and Johnstone, 1977; Eckert et al., 1982), although this approach will generally need to account for the lag period between infection and detection (even if this is attempted early in infection—for example, by fine slicing of liver tissue). Investigation of the prevalence of infection in lambs culled at different ages and inspected microscopically for infection gave useful information regarding the efficacy of a control scheme in south Powys, Wales (Lloyd et al., 1991; Gemmell et al., 2001a), and it has also been suggested that cattle may be good sentinel hosts for *E. granulosus*, due to the higher specificity of cyst detection in this species (Temple et al., 2013).

6.3. Surveillance in definitive hosts

Due to the asymptomatic nature of infection in definitive hosts, surveillance will generally be an active process based upon surveys, censuses and field trials. It has been advised by the WHO and the OIE that coproantigen testing is considered as the main method of diagnosis of canine echinococcosis during surveillance in the face of a control programme (Gemmell et al., 2001a). Currently, coproantigen ELISA data are generally interpreted in a dichotomous fashion—in order to differentiate test negative and test positive dogs, and estimate the overall coproantigen prevalence. The coproantigen prevalence would be expected to relate broadly to the prevalence of canine infection in a community, and can therefore act as a useful approximation of the overall levels of transmission and the potential risk to humans. However, data on the worm burdens of individual dogs may also be of importance due to the overdispersed nature of canine infection resulting in the majority of parasite biomass being concentrated in a relatively small number of infected dogs (Roberts et al., 1987). As a result of this, data on the distribution of worm burdens amongst dogs in a community are particularly useful for mathematical modelling of *E. granulosus* transmission (Torgerson, 2006b) and may be useful for the accurate estimation of rates of infection or reinfection during a control scheme (Torgerson and Heath, 2003; Atkinson et al., 2013). It is hoped that developments in the interpretation of ELISA results and use of real time PCR techniques (Knapp et al., 2014) may facilitate the estimation of worm burden from normal faecal samples, and therefore possibly allow fuller interpretation of data collected during routine surveillance.

One challenging aspect of definitive host surveillance is the selection of individuals to sample, as a sampling frame of dogs in a community will generally not exist. Dog registration (at the village, regional or country level) has been suggested where logistics are amenable to this (Gemmell et al., 2001a), and has been used successfully in a number of control schemes (Craig and Larrieu, 2006). However, this may not be feasible in remote areas, or situations where dog ownership is not clear: for example where community-owned dogs occur (Baronet et al., 1994). Attempts have been made in some cases to collect full census data for all (owned) dogs in an area of interest, but many approaches have been based upon the

collection of a convenience sample of owned dogs. Whilst this may be unavoidable, the limitations associated with this (especially if it results in the exclusion of unowned/stray/village dogs) should be considered when interpreting the results of surveillance.

6.4. Surveillance in nomadic populations

Nomadic and semi-nomadic populations present a particular challenge for echinococcosis surveillance (Macpherson, 2001). As these communities are often disconnected to some degree from the usual surveillance streams used, i.e. abattoirs, hospitals, veterinarians etc. (Zinsstag et al., 2006), most surveillance is necessarily active—based upon surveys. Surveillance of livestock echinococcosis is relatively rarely conducted in nomadic communities due to the challenges associated with obtaining a suitable sample size (Macpherson, 2001)—although the recent increase in use of ultrasonography in small ruminants offers a promising avenue for future work (Dore et al., 2014). Surveillance of human echinococcosis in nomadic or semi-nomadic populations has been greatly facilitated by the development of portable ultrasonography and to a lesser extent by rapid ELISA tests (Rogan et al., 1991; Macpherson, 2001), and canine echinococcosis surveillance has been improved by the development of molecular diagnostic tools such as coproELISA and coproPCR (Buishi et al., 2006).

6.5. Surveillance in animals during a control scheme

Whilst the issues described above are applicable to all of the three main uses of *Echinococcus* surveillance, there are some particular issues worth considering when surveillance is being used in the face of an intervention or control campaign. These will be affected to some degree by the intended aims of the campaign—for example, whether it is to eliminate CE as a human health issue; to reduce the level of *E. granulosus* infection in dogs; or to totally remove *Echinococcus* from the area in question (elimination). Despite the variety of specific aims of a control scheme, the general aim of an *Echinococcus* control scheme will be to reduce the prevalence of echinococcosis (whether this is in humans, definitive hosts, intermediate hosts, or a combination of these). Bearing this in mind, a general control scheme can be viewed as comprising one or more of four defined stages: described as 'planning', 'attack', 'consolidation' and 'maintenance of eradication' (Gemmell and Schantz, 1997; Craig and Larrieu, 2006).

During the planning phase, it is important to consider the available resources and data streams and to collect baseline data prior to the start of the campaign. In control schemes based upon treatment of dogs with praziquantel, the age- and frequency-distribution of cysts in intermediate hosts can be used to parameterise mathematical models in order to estimate the force of infection from dogs (Roberts et al., 1987; Ming et al., 1992; Cabrera et al., 2002). Similarly, estimates of the force of canine infection or rate of canine reinfection (e.g. from a pilot dosing scheme, or during the initial attack phase of the control scheme) from intermediate hosts can be estimated using mathematical models (Cabrera et al., 1995; Budke et al., 2005a; Torgerson, 2006b; Ziadinov et al., 2008). Ideally, quantitative estimates of the worm burden should be obtained—meaning that necropsy or purgation are currently the preferred diagnostic approaches. As well as establishing a baseline for ongoing surveillance, these outputs can help to identify the optimal frequency of praziquantel dosing for the communities in question. Surveillance of infection in wild and feral animals, in order to establish their role in the transmission cycle (and therefore whether they should be included in the intervention), would also be advised at this stage (Gemmell and Schantz, 1997).

During the attack phase, most surveillance is generally focussed on the measurement of the rate of reinfection in dogs. Coproantigen testing has been suggested to be appropriate for these studies, since exact estimates of the frequency distribution of worms may be less important for interpretation (Gemmell and Schantz, 1997; Moss et al., 2013), and some mathematical modelling of the force of infection using prevalence data may be possible (Ziadinov et al., 2008). Although often ignored, surveillance of infection in intermediate hosts is of importance during the attack phase—with sentinel surveillance in young animals being of particular use (Lloyd et al., 1991).

During the consolidation and maintenance of an eradication campaign (along with the later stages of the attack phase), an effective control scheme should have reduced the incidence of echinococcosis (i.e. the rate of new infections) to low levels. This should also relate to a decrease in the prevalence (the proportion of animals currently infected) in the definitive host, as well as in the intermediate host to some degree (given that the intermediate hosts are relatively short lived compared to the length of the attack phase). This situation can lead to difficulties in the interpretation of any diagnostic test with an imperfect specificity, as false positive test results (i.e. animals which test positive despite being uninfected) will become more common as the proportion of true negative animals increases (Schantz, 2006; Torgerson and Deplazes, 2009). There may also be a reduction in the sensitivity of the diagnostic test applied to dogs if the mean worm burden in the infected dog population is decreased so that infected animals may be missed when purgation or necropsy is used, or lower coproELISA OD values are found during coproantigen testing (Deplazes et al., 1992,1994; Varcasia et al., 2011). These issues make widespread diagnosis of infection in the definitive host less useful. A similar effect may be seen in intermediate hosts, as a reduction in the infection pressure from dogs could lead to a later onset of infection, which could reduce the sensitivity of abattoir surveillance (due to smaller cysts at presentation, and/or fewer older animals being processed by the abattoir system). Despite this issue, surveillance during the consolidation and maintenance phases is largely based on the detection of infection in intermediate hosts, due to the relatively high specificity of abattoir surveillance (especially if combined with molecular confirmatory testing), and the long-term persistence of cysts once animals are infected. This should be combined with animal tracing techniques in order to target ongoing surveillance (and possible control measures) to suspected areas of increased infection pressure (Gemmell and Schantz, 1997). As part of this targeted surveillance strategy, coproantigen testing of dogs (including unowned/stray/village dogs) may be beneficial within the identified 'high risk' areas (Economides and Christofi, 2000; Christofi et al., 2002).

6.6. Parasitological data interpretation for echinococcosis

Collection of parasitological data (i.e. the identification of parasites and/or parasite eggs rather than antigens, antibodies or DNA) is currently the mainstay of diagnosis of echinococcosis in intermediate hosts, and prior to the development of biological assays for the detection of infection was also the mainstay of diagnosis in the definitive host. However, the limitations associated with the collection and interpretation of parasitological data from definitive hosts mean that alternative approaches such as coproantigen ELISA or coproPCR are now more commonly used for diagnosis in these hosts.

Despite this, parasitological data remain an integral component of many studies of echinococcosis. One particular strength of parasitological data is that diagnosis of infection is generally of high specificity and, in conjunction with PCR methods, can allow genotyping down to the strain level (or even further, for molecular epidemiological studies). Whilst coproantigen PCR can also allow genotyping, there are a number of challenges associated

with extraction of DNA from faecal samples due to the presence of inhibitory substances. Secondly, collection of parasitological data often allows some estimate to be made of the parasite burden, which is an important consideration when constructing mathematical models of transmission due to the overdispersed nature of infection. Mathematical models can be of particular use in estimating the force of infection in either intermediate and definitive hosts (Cabrera et al., 1995; Torgerson et al., 2003a; Torgerson and Heath, 2003; Budke et al., 2005a; Ziadinov et al., 2008); for modelling the possible effect of control schemes (Torgerson, 2003, 2006b), and for identifying important characteristics of parasite ecology which would otherwise not be identifiable (Crofton, 1971; May, 1976; Roberts et al., 1986). A more detailed description of the creation and use of mathematical models of echinococcosis is beyond the scope of this article, but reviews are available elsewhere (Torgerson and Heath, 2003; Torgerson, 2006b; Atkinson et al., 2013).

6.7. CoproELISA interpretation in canine echinococcosis

As mentioned earlier, interpretation of coproantigen ELISA data has to date been based upon the determination of a single 'cut-off' optical density value which represents the frontier between negative samples (those with an OD value lower than the cut-off) and positive samples (with OD values higher than the cut-off). Whilst this approach provides an easily understandable result, and can be used to estimate the coproantigen prevalence in a community, it may suffer from a relatively low sensitivity and specificity. A commonly used approach for selection of an appropriate cut-off point is the 'Gaussian distribution method' (Allan et al., 1992; Deplazes et al., 1992). The aim of this approach is primarily to identify negative samples, which are assumed to follow a Gaussian distribution. A cut-off is selected which will correctly classify most negative samples, and is commonly selected as the point two or three standard deviations above the mean of a known panel of negative samples (often taken from a non-endemic area). According to the properties of the Gaussian distribution, this approach should result in correct classification of 99.9% of all negative samples (giving a specificity of 99.9%). Although no direct account is made for the distribution of positive samples, an attempt is made to minimise the area of overlap in OD values between negative and positive samples during the selection of diagnostic antibodies for the test by maximising the signal:noise ratio. However, the lack of any method of accounting for the distribution of positive samples in relation to the negative panel remains a potential problem with this approach. Additionally, it would not be a suitable approach in cases where the distribution of negative samples does not follow a Gaussian distribution (for example, due to cross reactions with other taeniid spp.).

One other complication associated with interpretation of coproantigen ELISA data is that the sensitivity of the test may vary according to the distribution of parasites in the community, since it has been well reported that animals with lower parasite burdens will tend to have lower OD values (Jenkins et al., 2000; Buishi et al., 2005a). Therefore, in a highly endemic situation, it would be expected that the test sensitivity would be higher than a situation in which all the infected animals have low worm burdens. Some attempt has been made for this by suggesting that the ELISA test is considered to have a 'threshold of detection' for worms (often suggested to be in the order of 50 worms), but as this threshold is not implicitly estimated using the Gaussian cut-off approach, this statement is difficult to validate.

Alternative methods of cut-off estimation are available which explicitly account for the distribution of negative and positive samples, although they appear to be relatively rarely used for interpretation of coproantigen ELISA data. The 'Youden index' (Youden, 1950) is based upon the selection of a cut-off which maximises both the test sensitivity and specificity (the actual Youden index is calculated as the sum of the sensitivity and specificity at the cut-off point which maximises both of these, minus one). Receiver operator characteristic (ROC) curve analysis is an extension of this principle which is based on the investigation of the effect of varying the cut-off point on two test 'operating characteristics': the sensitivity and the proportion of false positives, i.e. one minus the specificity (Zweig and Campbell, 1993; Greiner et al., 2000). If a non-parametric approach to ROC curve analysis is used, maximising the combined sensitivity and specificity estimates will give the same cut-off as the Youden index. However, one advantage of ROC curve analysis is that the cut-off can be tailored to the particular requirements of the test. Another advantage is that a measure of the overall discriminatory ability of the test (regardless of the cut-off selected) can be obtained by estimating the area between the ROC curve itself and the line of equivalence (where the sensitivity is equal to the proportion of false positives) (Swets, 1988). Despite the potential advantages of the Youden index and ROC curve approaches, one major challenge is the selection of an appropriate positive panel of samples—as the worm burdens within these samples would be expected to affect the OD distribution, and therefore could affect the optimal cut-off chosen.

More recently, alternative approaches towards interpretation of coproantigen ELISA data have been developed. These include methods based on interpretation of data following dichotomous classification in conjunction with the results of other tests such as PCR and purgation (Ziadinov et al., 2008; Hartnack et al., 2013), and methods which avoid the selection of a cut-off altogether. It is hoped that these approaches will maximise the information which can be obtained from coproantigen ELISA testing in the future.

7. Conclusions

The World Health Organisation includes cystic echinococcosis on a list of Neglected Zoonotic Diseases (NZDs) for which efforts to significantly reduce transmission by 2020 are to be prioritised; furthermore echinococcosis has also been listed as one of the world's 17 main Neglected Tropical Diseases (NTDs) (WHO, 2010a,b). Diagnosis of *E. granulosus* in domestic dogs and in livestock is a prerequisite for undertaking epidemiological studies, to carry out surveillance in endemic areas and to monitor efficacy of echinococcosis control programmes. Traditional approaches such as meat-inspection in slaughterhouses and necropsy examination of unwanted dogs are considered gold-standards, but are not perfect and not always be practically feasible, especially in remote and/or semi-nomadic communities.

The main advance in diagnosis of echinococcosis in animals over the last 20 years has been in the development of laboratory based high throughput coproantigen ELISAs for canine echinococcosis. CoproELISA has effectively replaced the use of arecoline purgation in many situations, and in association with PCR tests for DNA detection has enabled identification of the parasite to species and sub-species levels. The latter molecular approaches have also opened up the field of echinococcosis transmission in wildlife hosts. Practical obstacles still remain, however, in devising appropriate sampling strategies, sample collection, transport and timely cost-effective testing of faecal samples; also for coproELISA in optimal data management, analysis and interpretation. For detection of CE in intermediate hosts, meat inspection remains the gold-standard, though the advent of PCR now provides a tool for potential species-specific identification of small lesions in infected carcasses. Meat inspection, however, is still not close to being replaced by pre-mortem serodiagnostic methods. Alternatively, application of portable high resolution ultrasound scanning for detection of ovine/caprine CE shows promise with both ease-of-use and good levels of sensitivity and specificity.

Human CE remains predominantly a public health problem in resource-poor pastoral areas that are difficult to access, lack central slaughter facilities and may have dispersed human populations (Zinsstag et al., 2006; Craig et al., 2007). Control schemes to reduce the transmission of *E. granulosus* have been effective in some regions but required long periods and reliance on abattoir data for primary surveillance in animals. In rural resource-poor areas, surveillance of hydatid control programmes is now moving to predominant use of coproELISA to monitor dog infection levels following dosing schemes, in conjunction with mass ultrasound screening of human populations (Wang et al., 2001; Lembo et al., 2013). It is important however to understand that the predictive value of diagnostic tests will decrease as prevalence in animal hosts is reduced as a result of interventions, meaning that the optimal diagnostic strategy will change during the course of a control scheme.

Current research gaps in animal diagnostics include the need for robust commercially available coproELISA with higher sensitivity and species specificity (in particular, in order to differentiate *E. granulosus* (s.l.) from *E. multilocularis* in Eurasia), practical low cost copro-methods (coproantigen, coproDNA using PCR and/or qPCR) for rapid on-site detection of canine echinococcosis, better livestock surveillance at meat inspection with rapid PCR confirmation where needed, and thorough assessment of the use of ultrasound scanning for ante-mortem detection of ovine CE.

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