Risk factors for *Taenia saginata cysticercus* infection in cattle in the United Kingdom: a farm-level case-control study and assessment of the role of movement history, age and sex.

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Abstract

Bovine cysticercosis is caused by *Taenia saginata cysticercus*, the larval stage of the human tapeworm *Taenia saginata*. Recent European initiatives have highlighted the poor sensitivity of current surveillance for this parasite in cattle at slaughter; calling for more targeted, risk based and cost effective methods of *T. saginata cysticercus* detection. The aim of this study was to provide evidence that could inform such improved meat inspection activities in the United Kingdom (UK). The study included three components: (i) a farm-level case control study; (ii) the characterization of the network of movements of *T. saginata cysticercus* infected and non-infected animals, and an assessment of the strength of association between having passed through a farm that had previously originated an infected animal and the risk of infection; (iii) the assessment of the relationship between bovine age and gender and risk of infection. Abattoir records and cattle movement history data were used to identify farms of likely acquisition of infection (case farms) and a suitable control group. A questionnaire was used to gather farm-level characteristics and logistic regression was carried out to identify farm-level risk factors for the production of cattle found to be infected at slaughter. The case-control study provided evidence that farms situated close to a permanent potential source of human faecal contamination, and farms which used manure from animals other than cattle, were at higher risk of producing cattle later found to be infected with *T. saginata cysticercus* at slaughter. No other farm characteristics were identified as a risk factor for this. Analysis of the networks of animal movements showed that some individual farms played a key role as a source of *T. saginata cysticercus* infection; it was estimated that cattle with a history of being on a farm which previously appeared in the movement history of an infected animal were 4.27 times (P <0.001; 95%CI: 3.3-5.52) more likely to be diagnosed with *T. saginata cysticercus* infection at meat inspection. Male cattle aged 20 months or younger at the time of slaughter were found at lower risk of *T. saginata cysticercus* infection by comparison to other sex or age groups of cattle. These results, in combination with the consultation of experts and stakeholders, led to the conclusion that abattoir-based surveillance in low *T. saginata cysticercus* prevalence settings, such as Great Britain, could be made more targeted by stratifying cattle based on their individual movement history, sex and age characteristics.

Keywords

Bovine cysticercosis; *Taenia saginata cysticercus*; Risk factors; Case-control study; Targeted surveillance; Meat Inspection**.**

1. Introduction

Bovine cysticercosis is caused by the larval stage of the human tapeworm *Taenia saginata*. Humans are the definitive host and hold the adult tapeworm (taeniosis), while cattle act as the intermediate host and harbour the larvae (cysticercosis). Humans become infected after ingestion of raw or undercooked beef containing infective cysticerci (Dorny et al., 2010). The disease does not typically cause major health problems in humans, being characterised by mild symptoms, if any. Specifically, the adult tapeworm may cause mild inflammation at the site of its implantation on the intestinal wall, but substantial damage is generally not incurred. Nevertheless, the active discharge of proglottids from the anus, during the reproductive stage of the parasite, can cause emotional stress and an unpleasant sensation, making it unacceptable in most countries of the European Union (EU) (Dorny et al., 2010).

Cattle become infected through ingestion of food or water that is contaminated with human faeces containing viable *T. saginata* ova. It has been shown that these eggs can remain viable for several weeks or months in sewage, water or on pasture (Allan et al., 2005). Within the bovine intestine, each egg may release an oncosphere which, over a period of 8-10 weeks, migrates through the haemolymphatic system to establish in skeletal or cardiac muscle (and less commonly in fat or the visceral organs) as an infective cysticercus (Herenda and Chambers, 1994). These cysticerci (viable cysts), remain infective for approximately nine months before they eventually die and calcify, becoming non-infective (non-viable cysts) (Allan et al., 2005). Both viable and non-viable cysts can be present in the same animal.

The national prevalence of *T. saginata cysticercus* in cattle at individual animal level, based on post mortem inspection, has recently been quoted at low levels between 0.01% and 0.06% in European countries (Dorny et al., 2010; Calvo-Artavia et al., 2013e). However, the high variability of *T. saginata cysticercus* prevalence within and between European countries, with some national estimates ranging up to 6.2%, has also been described (Laranjo-González et al., 2016). The most recent UK-specific estimates describe a low prevalence; official government study results relating to cattle slaughtered between 2008 and 2011, state that 15 out of 190,493 calves (0.008%) and 2,674 out of 8,484,371 adult cows (0.032%) were found to be infected with *T. saginata cysticercus* at meat inspection (MLCS, 2013; Hill et al., 2014).

The presence of *Taenia saginata* cysts in cattle is determined during post-mortem meat inspection and enforced in the EU through Regulation (EC) No 854/2004 of the European Parliament and of the Council. This current slaughterhouse regulation requires that meat inspectors evaluate all bovine carcases above six weeks of age, using the following palpation and incision techniques: (1) visual examination and palpation of the tongue (2) two deep incisions in the external masseter muscle and one deep incision in the internal masseter muscle, parallel to the mandible (3) visual examination of the heart (incised lengthwise to open ventricles and to cut through the interventricular septum (4) visual examination of the diaphragm and oesophagus.

Carcasses with visible cysts (independent of the method of detection at meat inspection) are either downgraded (requiring extra handling and freezing to inactivate the cysticerci) or condemned, depending upon the amount, location and type of visible cysts (whether they are infective or non-infective). Whilst this process is necessary for the protection of public health, it incurs significant costs (Dorny et al., 2010). The sensitivity of visual inspection is assumed to be low (<30%) and positively correlated with the amount and size of the cysts (Dorny and Praet, 2007; Dorny et al., 2010; Allepuz, 2012). Expert consultation has suggested that the sensitivity of visual inspection may also be dependant on the type of cyst, with non-viable cysts being easier to identify than viable cysts through tissue incision and palpation, due to their differing textures. This discrepancy may have an added impact on public health, since it is the viable cysts that are considered infectious to humans. Thus, the need to develop more sensitive tests has been recognised (Dorny et al., 2010). However, development and accreditation of a more sensitive test (such as PCR) is time consuming and its application may not be practical or cost effective under the current meat inspection system (Dorny et al., 2010).

Currently, European legislation regarding the meat hygiene inspection system is under review. It has been suggested that the stratified sampling of cattle can be appropriate where the national prevalence is low (<0.1%) (Dorny et al., 2010) and simulation models suggest that meat inspection systems integrating this sampling technique could potentially be more cost-effective, without compromising on public health (Calvo-Artavia et al., 2013a). Thus, in addition to highlighting the poor sensitivity of meat inspection methods, the most recent European Food Safety Authority (EFSA) report (Dorny et al., 2010) also emphasizes the need for more targeted, risk-based and cost-effective methods of *T. saginata cysticercus* (also known as *Cysticercus bovis*) surveillance in cattle at slaughter.

Based upon these recommendations the aim of this study was to provide evidence that could inform more targeted meat inspection activities in cattle at slaughter in the UK. The specific objectives were (1) to identify farm-of-origin characteristics associated with the risk of animals being found infected with *T. saginata cysticercus* at meat inspection and (2) to assess whether the age, sex and movement history of animals found to be infected were different from that of control animals not found to be infected at meat inspection.

2. Material and methods

The study included three components: (1) a literature review and a case-control study with the farm as the unit of interest; (2) characterization of the network of movements of infected and non-infected animals, including an assessment of the strength of association between having passed through a farm that had previously originated an infected animal and the risk of infection; (3) assessment of the relationship between age and gender and risk of infection.

2.1. Case-control study

2.1.1. Selection of case and control farms

The individual slaughter records were first collected for all cattle found to be infected with *T. saginata cysticercus* at any of the 197 approved slaughterhouses across the UK between January 1st 2013 and January 31st 2015. The movement history for these animals was obtained from the British Cattle Movement Service (BCMS) Cattle Tracing System (CTS) database for those animals slaughtered in GB; or from the Department of Agriculture and Rural Development of Northern

Ireland (DARD NI) database for those slaughtered in Northern Ireland (NI). The movement history for each infected animal entailed the identification and addresses for all farms resided upon over the animal's lifetime. Other holdings such as markets were removed from the movement histories, as farms were the holdings of interest. Case farms were then selected from the combined movement histories pertaining to these infected animals. Case farms were defined as follows: (1) a case farm is the only farm cited in the movement history of an infected bovine or (2) a case farm is not the only farm cited in the movement history of an infected bovine, but it is cited in the movement history of at least one other infected bovine or (rarely) it is cited more than once in the movement history of an infected bovine. In parallel, the same information was collected for a comparison group of animals diagnosed as not infected with *T. saginata cysticercus* at meat inspection. Three of these 'uninfected' animals were selected per infected animal, matched by slaughter house and slaughter date, to enable the most cost-effectively achievable statistical power in later analyses. For 99.6% of the uninfected animals the full lifetime movement history for each individual was available (as it was for all infected animals). For the remaining 0.4% of uninfected animals the movement history only covered a period of up to three years preceding slaughter; such incomplete movement history records may have belonged to animals imported direct to slaughter, for example. All movement history records were then restricted as described for infected animals. The records for animals that were imported direct to slaughter were also removed in this process. Control farms were identified from the combined movement histories of these uninfected animals and were defined as follows: (1) a control farm is the only farm cited in the movement history of an uninfected bovine and is not found in the movement history of any infected animal in the dataset; (2) to increase the number of control farms available for selection (and enable a 1:3 ratio of case to control farms) control farms selected through criterion (1) were combined with a random sample (created via Microsoft Excel random number generator) of those selected via the following criterion: a control farm is not the *only* farm cited in the movement history of an uninfected bovine, but it is found only in the combined movement histories of all uninfected animals in the dataset and it is not found in the movement history of any infected animal in the dataset. As per the individual cattle that they related to, control farms were therefore in a 3:1 ratio to case farms. In line with these definitions, data queries were enlisted within Microsoft Access in order to select case and control farms from the collective movement histories.

2.1.2. Collection of data on farm-level risk factors

2.1.2.1 Identification of farm-level risk factors

To identify putative farm-level risk factors, a review of published evidence was performed on three databases (Pubmed, CAB abstracts and ScienceDirect). The following search terms were specified for, within the title of search result publications: ((bovine cysticercosis) OR (Cysticercus bovis) OR (C. bovis) OR (Taenia saginata) OR (taeniosis)) AND ((inspection) OR (risk-based) OR (risk factors) OR (husbandry) OR (practice) OR (surveillance) OR (indicators)). References were considered for inclusion within the review if they had been published in English, between 2000 and 2014. Confirmation of the adequate identification of risk factors was provided by consultation of experts in the field of *T. saginata cysticercus* research.

2.1.2.2 Questionnaire design and administration

Each of the case and control farms selected received a postal questionnaire, designed to collect information relating to a range of farm characteristics. Initial questionnaire packs, and subsequent staggered reminder questionnaire packs, were posted to all selected farms during May and June 2015. All questionnaire packs contained an initial or reminder letter of introduction, detailing information about the project, in addition to the questionnaire itself, with a backing page requiring signed and dated consent to use the farmer’s answers as per the project outcomes. Farmers were also given the alternative option of completing an online electronic version of the questionnaire. Farmers were awarded a voucher on return of their completed questionnaire. Replies were received over an open period of two months following postage of reminders.

The questionnaire contained 134 questions in English, spanning the farmer's identity and role on farm, general cattle management practices on farm, feeding and water systems on farm, transport and destinations for animals leaving the farm, farm land management practices and the interactions between cattle and people visiting or working on the farm. The majority of these questions were focused on those farm characteristics found, or theorized, to be associated with greater risk of *T. saginata cysticercus* infection in cattle, according to the systematic review of published literature (Table 1). Questions were mostly closed or semi-closed, with areas for additional comment; a small minority of questions were open-ended. The questionnaire was divided into five parts, with the first three referring sequentially to beef, dairy and hobby cattle production practices specifically; therefore most farmers were expected only to answer one of the first three sections. In order for questionnaires to be accepted as adequately completed, a distinct majority of questions within at least one of the first three sections, and within both of the last two sections, had to be answered. Signed and dated consent given by the farmer was a prerequisite for acceptance of completed questionnaires.

2.1.3 Data analysis

Information on farm characteristics derived from the adequately completed GB questionnaires was collated, cleaned and in some cases combined, re-categorised or excluded; based on the completeness of answers and a-priory relevance. Additionally, only characteristics that could be adequately combined across beef, dairy and dairy/beef production systems, with minimal effect of missing values, were included in the selection. This process resulted in a subset of data including 30 farm characteristics, which were employed as explanatory variables in the subsequent statistical analyses. Explanatory variables with few missing values were not excluded from this list. Logistic regression was then utilised to analyse the data (Dohoo et al., 2003), using the 'epicalc' package in R software (R Core Team (2015), R Foundation for Statistical Computing, Vienna, Austria). Univariable logistic regression analyses were carried out to assess the strength of association between the dependant variable (farm status: case or control) and each explanatory variable individually. Any explanatory variables for which the evidence of association with the dependant variable was described by a P value of <0.2 were selected for the second step of the analysis, where multi-collinearity between explanatory variables was assessed through additional univariable logistic regression analyses. If collinearity was found only one of the two variables was retained for input into a multivariable model as the final stage of the analysis. Five explanatory variables passed through to the final stage. Multivariable logistic regression involving a stepwise forwards selection method was utilised to create the final model. Explanatory variables were retained when $P<0.05$ (by Wald test).

The geographic locations of case and control farms were visualised using ArcGIS 10.2.2 (ESRI ArcMap 2014). The spatial scan statistic using a Bernoulli purely spatial model was implemented in SaTScan™ version 9.4.2 to assess whether there was spatial clustering of case compared to control farms (Kulldorff and Nagarwalla, 1995). The size of the circular window was limited to 50% of the population at risk and the statistical significance of spatial clusters was assessed using 999 Monte Carlo simulations. Due to the differing data security requirements, addresses for NI farms were not available, therefore NI farms are not included in the distribution map (Figure 1).

2.2. Characterization and comparison of movement history between infected and non-infected animals

To build and compare the networks of animal movements of infected and uninfected animals, the movement histories of the infected animals slaughtered in GB, together with an equal number of the uninfected animals slaughtered in GB, were extracted from the data used for case and control farm selection. R version 3.1.1 'igraph' package (Csardi and Nepusz, 2006) was used to create directed networks based on the movement histories of these animals, where farms formed the nodes and animal movements comprised the edges. In-degree and out-degree were obtained for each farm as a measure of the number of cattle movements onto and off farm respectively (Freeman, 1979). Univariable logistic regression (Dohoo et al., 2003) using the 'epicalc' package in R software (R Core Team (2015), R Foundation for Statistical Computing, Vienna, Austria) was used to assess the strength of association between animal infection status (the dependant variable) and whether an animal slaughtered between the 1st January 2014 and the 31st December 2014 had resided on a farm previously resided on by an infected animal slaughtered in the previous year (between the 1st January 2013- 31st December).

2.3. Assessment of the relationship between cattle age and gender and risk of infection

Logistic regression (Dohoo et al., 2003) was also used to assess the relationship between sex and age at time of slaughter (less than 20 months vs. more than 20 months) and infection status of the individual animal (the dependant variable), using Stata 14 (StataCorp LP, College Station, Texas). The selection of two age groups with a cut-off of 20 months was made firstly since this reflects the age at which fattening calves are generally sent to slaughter (14-30 months), and secondly because increasing the number of categories would have resulted in very small numbers of animals in some groups, giving poor potential for comparison.

3. Results

The literature search yielded a total of 77 relevant papers providing information on risk factors for *T. saginata cysticercus* infection in cattle, these are summarised in Table 1 and were generally related to cattle residing in close proximity to humans or human effluent.

Table 1. Previously reported risk factors for the infection of cattle with *T. saginata cysticercus;* from a review performed on three databases (Pubmed, CAB abstracts and ScienceDirect), concluded in January 2015.

Risk Factors	Reference(s)

Water supply for animals Organic wastes as fertilizers Roughage types (hay, silage, or crop by-products (e.g. potato by products), originating from locations contaminated with human waste) Farm location Direct on-farm human excrement deposition Staff training and turnover Calf age	(Dorny et al., 2010)
Outdoor defecation in or near cattle rearing facilities or pastures Lack of effective fly and bird control around cattle facilities Use of sewage effluent, sludge or untreated human faeces to irrigate or fertilize feed crops and pastures Human carriers involved in the rearing and care of cattle Indiscriminate deposition of faeces on campgrounds, along highways, and along rail tracks	(Allan et al., 2005)
Flooding of pastures Free access of cattle to surface water Proximity of wastewater effluent	(Boone et al., 2007)
Presence of a railway line or a cark park close to areas grazed by cattle Leisure activities around areas grazed by cattle Use of purchased roughage Organized public activities on farms attracting visitors	(Flutsch et al., 2008)
Bovine gender Bovine age Grazing Access to risky water sources with sewage treatment plant effluent in proximity Sharing machinery or hiring contractors	(Calvo-Artavia et al., 2013a; Calvo-Artavia et al., 2013c; Calvo-Artavia et al., 2013e)
Bovine age Bovine gender	(Dupuy et al., 2014)

A total of 648 bovines (0.013%) were diagnosed as infected with *T. saginata cysticercus* in UK approved abattoirs, out of an estimated 5.1 million cattle slaughtered in the UK between January 1st 2013 and January 31st 2015 (DEFRA, 2014). An individual animal was defined as infected with *T. saginata cysticercus* based on data derived from its meat inspection record. Thus, an infected animal was one that was recorded as a positive for *T. saginata cysticercus*, with further information given on the type of cyst identified (a viable cyst or non-viable cyst diagnosis) or was recorded as a positive for *T. saginata cysticercus* with no further detail stated on the type of cyst identified (a positive diagnosis), see Table 2.

After combining the movement history data for these animals, the study sample from which case and control farms were selected contained a total of: 2270 cattle slaughtered in GB, comprising 592 infected bovines and 1678 uninfected bovines; and 141 cattle slaughtered in NI, comprising 36 infected bovines and 105 uninfected bovines. The distribution of the GB sub-sample, by sex, age group and *T. saginata cysticercus* diagnosis is presented in Table 2.

Table 2. Distribution of the sample of cattle slaughtered in GB between 1st January 2013 and 31st January 2015, by sex, age and *T. saginata cysticercus* diagnosis.

Diagnosis	Male		Female		Total
	0-20 months	21-194 months	0-20 months	21-294 months	
Viable cyst	8	43	5	74	130
Non-viable cyst	36	160	33	197	426
Positive	3	6	2	25	36

Uninfected	360	506	102	710	1678
Total	407	715	142	1006	2270

A total of 233 case farms (219 from GB and 14 from NI) and 712 control farms (666 from GB and 46 from NI) were identified across the UK.

The geographic locations of GB case and control farms are presented in Figure 1. This distribution of case and control farms probably reflects underlying patterns in the GB cattle farm population. No statically significant spatial clusters were detected.

Out of the total 885 GB case and control farms selected and sent questionnaires, 229 of them (26%) returned adequately completed questionnaires. GB hobby farms (3 questionnaires received, 1 of them from a case farm) and all farms from NI (11 questionnaires received, 1 of them from a case farm) were excluded from further analysis. Inadequately completed questionnaires were also received from GB (45) and NI (7) farms. Thus the final dataset contained 226 farms in total, comprised of GB beef, dairy and beef/dairy farms, of which 57 were case farms, and 169 were control farms (maintaining the 3:1 ratio of control to case farms).

Only one of the farm characteristics analysed: “Farmer uses own and/or bought in manure from animals other than cattle (including treated human sewage) for pasture fertilisation", was significantly associated with farm status in the univariable analysis (OR 2.66, 95% CI 1.09-6.47, P 0.03). The results of the final multivariable logistic regression model are presented in Table 3.

Table 3. List of explanatory variables which were found to be significantly associated with a farm being a case farm within the final multivariate model. Data relating to these variables was provided by 201 (49 case and 152 control) of the 226 GB cattle farms which returned adequately completed questionnaires (January 2013-2015).

Farm characteristic	% exposed among cases and controls	Crude odds ratio (95% CI)	Adjusted odds ratio (95% CI)	Wald's test P value
Farm close to a permanent potential source of human faecal contamination	40.8 % (cases) 29.6 % (controls)	1.77 (0.91,3.41)	2.04 (1.03,4.04)	0.041
Farm uses own and/or bought in manure from animals other than cattle	20.4 % (cases) 8.5 % (controls)	2.74 (1.12,6.73)	3.25 (1.29,8.22)	0.013

The final model included only two variables; farms which were situated close to a permanent potential source of human faecal contamination (e.g. sewage works) had a marginally higher odds (adjusted OR 2.04; 95% CI 1.03-4.04) of producing cattle infected with *T. saginata cysticercus* at slaughter. Farms which used their own and/or bought in manure from animals other than cattle to fertilize their pasture, which included treated human sewage, had a higher odds (adjusted OR 3.25; 95% CI 1.29-8.22) of producing cattle infected with *T. saginata cysticercus* at slaughter.

Analysis of the between-farm movement networks for cattle found to be infected with *T. saginata cysticercus* at slaughter and, separately, for those found to be uninfected, are presented in Figure 2, as the ‘case’ and ‘control’ network respectively. The case network comprised 899 farms, whilst the control network comprised 875 farms. The range of in-degree in the case network was 0-49, whilst it was 0-18 in the control network. In the case network 6 farms received animals from more than 10 farms, however in the control network there was only one such farm.

Among the 2262 animals within the dataset used for the network analysis, 40% of the infected animals and 14% of the uninfected animals slaughtered in 2014 had inhabited a farm in which an animal, diagnosed as infected at slaughter in 2013, had previously resided. Those animals with a history of being on a farm which previously appeared in the movement history of an infected animal had 4.27 times higher odds of being diagnosed infected at slaughter ($P < 0.001$; 95% CI: 3.3-5.52).

An interaction was found between age and sex and risk of infection. Using the sex-age category associated with the lowest odds of *T. saginata cysticercus* infection in cattle (males of 0-20 months of age at slaughter) as a reference, females between 0-20 months were found to have a 3 times higher odds of infection at slaughter (OR 3.00; 95% CI 1.87-4.83); males of 21-194 months were found to have a 3.16 times higher odds of infection at slaughter (OR 3.16; 95% CI 2.24-4.46); and females of 21-194 months were found to have a 3.19 times higher odds of infection at slaughter (OR 3.19; 95% CI 2.29-4.45). Within the younger age group of 0-20 months, males exhibited a distribution skewed further towards the minimum age than females, as shown in Figure 3.

4. Discussion

Between 1st January 2013 and 31st January 2015, a total of 612 *T. saginata cysticercus* infected animals were identified in approved abattoirs in Great Britain. These results are indicative of a very low true prevalence of infection that would range between 0.043% and 0.086% for sensitivity values between 15% and 30% and 100% specificity. These values appear to be reasonable based on published literature (Abuseir et al., 2006; Dorny and Praet, 2007; Dorny et al., 2010; Allepuz, 2012). Movement history data were available and acquired from the British Cattle Movement Service (BCMS) for 98.5% of the GB cattle slaughter records acquired for this study. DARD NI provided movement history data for 100% of the NI cattle slaughter records. This degree of synchrony suggests that cattle movement data are very comprehensively recorded, and thus could be effectively utilised to support more targeted meat inspection activities in cattle at slaughter in the UK.

The case-control study of 226 GB cattle farms (57 cases and 169 controls) found some evidence of association between farm characteristics and the risk of *T. saginata cysticercus* infection: animals from farms which were situated close to a permanent potential source of human faecal contamination (e.g. sewage works), and farms which use their own and/or bought in manure from animals other than cattle to fertilize their pasture (which included treated human sewage) were at higher risk of *T. saginata cysticercus* infection. These findings concur with known biological facts regarding the life cycle of *T. saginata cysticercus*, or *Taenia saginata*, the adult stage of the parasite in humans (Allan et al., 2005). None of the other farm characteristics under study were found to be associated with the risk of infection, but caution should be taken when ruling out other farm-level factors as contributors to the risk of infection, as the power of our study was limited by the magnitude of the final farm sample obtained. Unlike Boone et al (2007), our study did not find evidence for an association between a farm being prone to flooding and the risk of infection, therefore, further investigation of geographic and meteorological factors was not undertaken. Evidence relating to farm characteristics as risk factors for *T. saginata cysticercus* infection in cattle is complex, highly variable and occasionally conflicting, as described by the results of our review. The low prevalence of *T. saginata cysticercus* in the UK and other parts of the EU (Dorny et al., 2010; Calvo-Artavia et al., 2013c) implies that isolated one-off events that place an individual farm or a small number of farms at high risk at a particular point in time may have a large contribution to the overall risk of infection at national level. This is illustrated by the 2013 outbreak in the UK (AHVLA, 2013, 2014) in which the source of *T. saginata cysticercus* infection was thought most likely to be potatoes from a single origin, that were used as cattle feed. Our results and the weak associations identified between farm characteristics and odds of infection in cattle at slaughter are compatible with a low prevalence situation and the sporadic emergence of new case farms. In this situation, where a clear demographic pattern is lacking, the discriminatory power of a targeted surveillance system for *T. saginata cysticercus* in cattle at slaughter, based on farm characteristics, would be limited. Additionally, information on farm characteristics are not currently routinely collected, and farm characteristics such as manure use for pasture fertilization are frequently dynamic (varying with the seasons on any particular farm, for example).

Therefore, instigating a reliable system for the recording of such characteristics across the entire UK farm population would likely prove exceptionally challenging.

While our results suggest that the potential for using farm characteristics to target *T. saginata cysticercus* surveillance activities may be limited, the exploration of the network of movements of infected and non-infected cows at slaughter suggest that some farms may play a key role as a source of *T. saginata cysticercus* infection and that movement histories may be useful for targeting abattoir inspection activities. Our results also concur with Flütsch, F. et al. (2008) who found that 29.4% of all case farms had previously experienced cases of Cysticercosis in Switzerland. However, it must also be taken into consideration that large farms moving large numbers of cattle onto and off the premises may be more likely to appear in the movement network of infected animals since they are more likely to buy them in.

Our results suggest that males of 20 months or younger are at a significantly lower risk of infection than other members of the slaughter population. In the UK, young males of 0-20 months tend to be dairy calves stored for fattening for beef, it is plausible that they are more commonly reared indoors and fed a diet largely comprised of concentrates rather than roughage. Additionally, since males of 0-20 months tend to be skewed towards younger ages than females within the same age bracket (see Figure 3), they are theoretically less likely to have been exposed to potential infection over their lifespan. Our results with regard to this concur with those of other studies, which found that female gender and old age were both risk factors for *T. saginata cysticercus* infection in cattle (Dupuy et al., 2014).

The pattern of an animal's movement history appears to have more discriminatory power than the characteristics of the individual contact farms. There is therefore potential for using movement history and previous *T. saginata cysticercus* diagnoses to target meat inspection at slaughter. Additionally, movement history information is also currently well standardised and readily available across UK cattle data collection systems. In relation to this, a workshop was held on 14th September 2015, to present the interim results of our analyses of farm characteristics and movement history to different stakeholders. The aim of this workshop was to promote discussion and formulate conclusions with regard to possible industry challenges related to the design, transfer and implementation of a targeted surveillance system for *T. saginata cysticercus* in UK cattle at meat inspection, based on the concept of a classification tool which would divide cattle into high and low risk groups, dependent upon their movement history characteristics. The conclusion from this workshop was that, for the above reasons, any proposal for a 'classification tool', based upon these results, should focus on grouping cattle based upon their movement histories and individual animal risk factors such as sex and age, rather than the characteristics of the farms in their movement histories.

When considering the overall results of this study, a number of limitations and assumptions must be taken into account. Firstly, since cysts are considered to be viable for a specified period of nine months, concentrating solely on these diagnoses could have made the selection of case farms more accurate. However, all positive diagnoses of *T. saginata cysticercus* were grouped together in this study, regardless of cyst type. In addition to gaining added reliability in results generated from a larger sample, this grouping was done for two main reasons. Firstly, since cysts are considered to be viable for a specified period of nine months, concentrating solely on these diagnoses could have made the selection of case farms more accurate. However, all positive diagnoses of T. saginata cysticercus were grouped together in this study, regardless of cyst type. In addition to resulting in a larger sample, this grouping was done for two main reasons. Firstly, viable cysts are difficult to diagnose through visual meat inspection and palpation since they resemble animal tissue. Secondly, infected animals may harbor multiple cysts at various stages (Scandrett et al., 2009). These two points could have lead to the misclassification of animals (and farms) if only those animals with viable cyst diagnoses were defined as 'infected'.

Additionally, the meat inspection records of infected animals did not always record the type of cyst found.

A related limitation is the potential for variation in the definitions of *T. saginata cysticercus* infection at meat inspection between abattoirs and meat inspectors, i.e. ‘positive’, ‘non-viable cyst’, ‘viable cyst’; which may have led to some misclassification of animals and farms in the study. Equally, due to poor sensitivity, a considerable proportion of truly infected animals are missed by current inspection methods, resulting in the potential inclusion of farms that produced infected animals as controls in our study. However, given the very low prevalence of infection this should have had minimal effect on the results.

The relatively high proportion (69%) of GB non-respondents within the case-control study could also have introduced bias if non-respondents systematically differ from respondents with respect to the farm characteristics studied. We made a conscious decision to create a more detailed questionnaire, in order to assess a large range of characteristics, in the knowledge that this would take longer for respondents to complete and potentially reduce the response rate. Additionally, selecting variables from the questionnaires, with subsequent coding and re-categorization pre-statistical analysis, was a highly subjective process, although based on previous literature and sound biological principles.

5. Conclusions

Variable and uncertain sensitivity of meat inspection precludes accurate estimation of the prevalence of *T. saginata cysticercus* in cattle in the UK and elsewhere; assuming a conservative (low) sensitivity of 15% no more than 0.086% of cattle slaughtered in Great Britain during 2013-14 were infected. Infected animals are more likely to come from farms which are close to a permanent potential source of human faecal contamination, and/or which use manure from animals other than cattle to fertilise their pasture. Cattle that have resided on a farm on which an infected animal (diagnosed at slaughter) has resided before them are at higher risk of infection. Compared to other age/sex groups, young male cattle are less likely to be diagnosed with *T. saginata cysticercus* infection at slaughter. Our findings therefore indicate that current surveillance in low *T. saginata cysticercus* prevalence settings could be made more targeted through grouping cattle based upon their individual movement history, sex and age characteristics. By contrast, farm characteristics appear to offer limited potential to fulfil this remit.

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Ethical Approval

The authors confirm that this study has been ethically reviewed by the Clinical Research Ethical Review Board (CRERB) affiliated with the Royal Veterinary College (RVC) and that ethical approval has been granted under reference (URN) number 2015 1341.

Conflicts of interest: none.

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Figure 1. The distribution of case farms (n=219, a)) and control farms (n=666, b)) included in the case-control study of risk factors for *T. saginata cysticercus* infection, in Great Britain.

Figure 2. Networks of between-farm movements of animals found at slaughter to be infected (case network) or uninfected (control network) with *T. saginata cysticercus* between 1st January 2013 and 31st January 2015; dots represent farms whilst arrows represent cattle movements.

Figure 3. Box and whisker plot of the age distribution of males and females within the age group of 0-20 months of age at slaughter.

Figure 1

a) b)

Figure 2

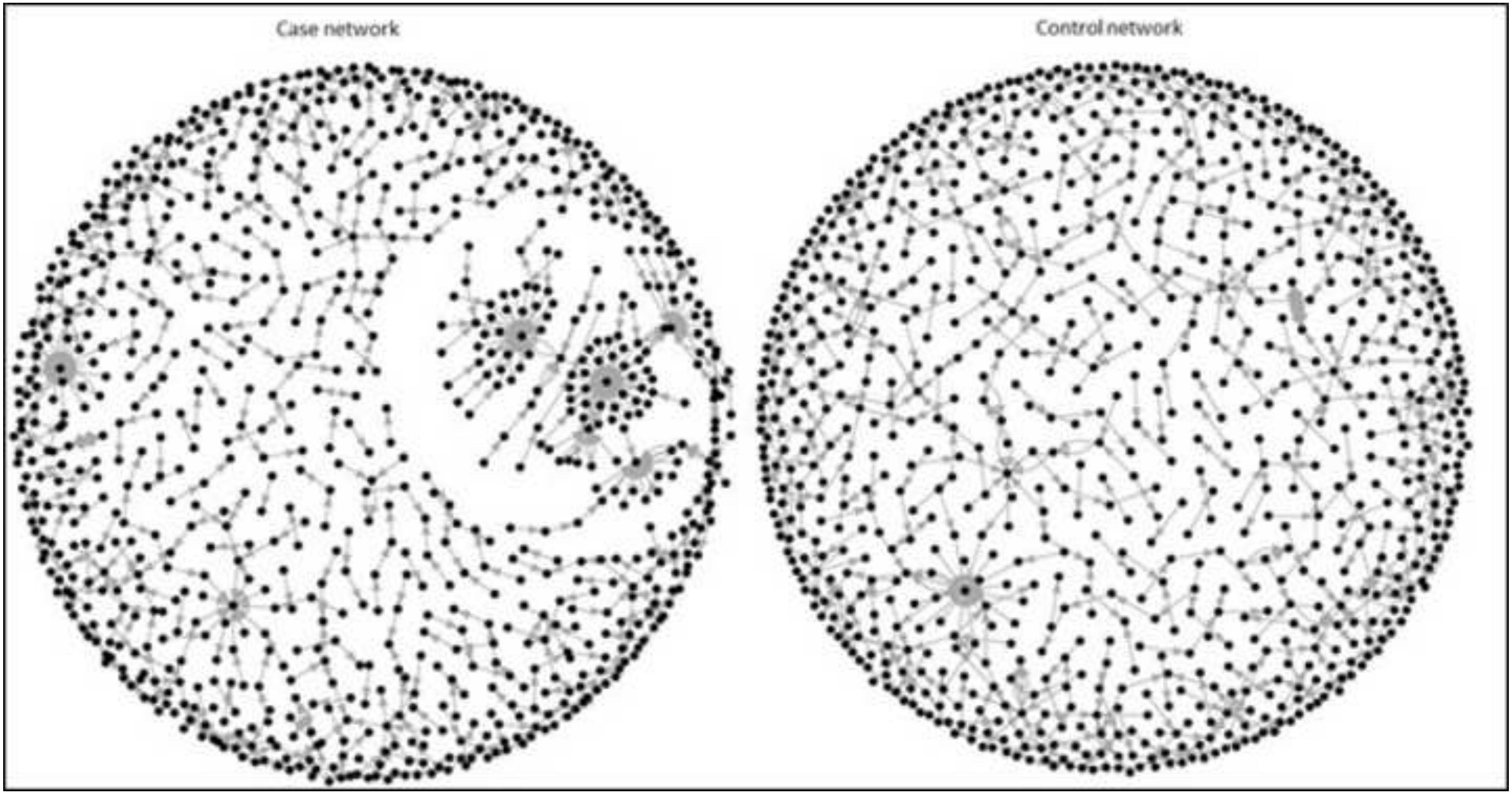

Figure 3