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Short communication

Toxoplasma gondii detection in cattle: A slaughterhouse survey

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ABSTRACT

Toxoplasmosis is a zoonotic disease caused by the protozoan parasite *Toxoplasma gondii*. Ingestion of raw or undercooked meat containing viable cysts has been suggested to be a major source of *T. gondii* infection in humans. Suboptimal performance of serological assays in cattle has traditionally precluded accurate quantification of the extent to which cattle populations are infected and their meat harbour tissue cysts. In the absence of accurate estimates of the level of infection in the animal population, assessments of likely human exposure through the consumption of cattle meat remain highly speculative. Following the development of novel and sensitive molecular methods that can be applied to the relatively large numbers of samples required in observational studies, the first quantitative estimates of the frequency of *T. gondii* in meat samples from naturally infected cattle have become available recently. Such estimates are critical for the development of quantitative risk assessment models that could be used to inform food safety policies. The aim of this study was to generate the first estimates of the prevalence of *T. gondii* infection in a sample of cattle exposed to natural levels of infection and slaughtered for human consumption in the UK under commercial conditions. Such estimates provide great value to the global assessment of *T. gondii* burden given the scarcity of data available on the frequency of natural infection in cattle populations worldwide.

Between October 2015 and January 2016 diaphragm samples were collected from 305 animals, slaughtered in ten commercial slaughterhouses across the UK. Movement histories showed that the animals sampled (41.6% females and 58.4% males) had passed through a total of 614 farms and 40 livestock markets across the country. Five animals (1.6%) were deemed positive for *T. gondii* following magnetic capture real-time PCR, confirmed by amplicon sequencing. The true prevalence of infection was estimated to be 1.79%. All positive animals were male, none of whom had been on the same farm and/or livestock market before slaughter and there was no apparent geographic pattern. The results from this study suggest a low level of infection in cattle raised and slaughtered in the UK and can be used to populate the first stages of formal risk assessments to quantify the likely extent of human exposure to *T. gondii* through the consumption of beef with relevance to the UK, EU and rest of the world.

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1. Introduction

Toxoplasmosis is a zoonotic disease caused by the apicomplexan parasite *Toxoplasma gondii* (Montoya and Liesenfeld, 2004). Domestic cats and other felids are the definitive hosts, while mammals and birds are the most common intermediate hosts. Oocysts produced in the definitive host are passed in faeces and sporulate in the environment before being ingested by an intermediate or another definitive host. When sporulated oocysts are ingested by an intermediate host sporozoites are released, infecting numerous tissues (predilection tissues), where they undergo endodyogeny to form tachyzoites. While predilection tissues vary between species, muscle, liver, brain and the intestinal epithelium are commonly infected (Dubey et al., 1998; Roberts and Janovy, 2005). Following infection, the parasite develops into tissue cysts where the parasite multiplies (termed bradyzoites at this stage).

The sero-prevalence of *T. gondii* infection varies between host species and country. It is estimated that up to 30% of the global human population is infected (Tenter et al., 2000). Ingestion of raw or undercooked meat containing viable cysts has been suggested to be a major source of *T. gondii* infection in some European coun-

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tries (Cook et al., 2000; Flatt and Shetty, 2012), however the relative contribution of different types of meats to human *T. gondii* infection is unclear. Herbivorous livestock are most likely to become infected from the ingestion of infective oocysts in the pasture, feed or drinking water (Andreoletti et al., 2007). In cattle, sero-prevalence estimates vary from 1% to 92% worldwide, however the results are not directly comparable given differences in study design and the test used. Crucially, sero-prevalence is indicative of exposure to the parasite, not cyst development. Contrary to sheep and goats, clinical signs are rarely exhibited in cattle (Dubey, 2010).

Reliable prevalence estimates in meat-producing animals are needed as the first stage in formal risk assessments aiming to estimate the relative contribution of meat to human *T. gondii* infection. The lack of information regarding the level of infection in cattle reared in the UK and Europe has been highlighted by the UK Food Standards Agency (FSA) and the European Food Safety Authority (EFSA) (AMCSF, 2012; Andreoletti et al., 2007). Routine detection of *T. gondii* cysts during meat inspection is not feasible given the microscopic size of the cysts. Instead, diagnosis of *Toxoplasma* infection most commonly relies on serological detection. Although numerous techniques are available for detection of antibodies, the lack of correlation between seropositivity in cattle and presence of detectable cysts has limited the value of serology as an indirect indicator for cyst occurrence in beef (Opsteegh et al., 2016b, 2011). Therefore, direct detection methods are necessary to provide estimates of the proportion of cattle harbouring cysts, a critical input for a sound assessment of the risk of human infection associated with the consumption of cattle meat.

The gold standard for detecting *T. gondii* in meat samples is bioassay using mice or cats. However, these methods are relatively expensive, very time consuming and not conducive for the screening of large sample numbers (da Silva and Langoni, 2001). More recently, molecular approaches such as polymerase chain reaction (PCR) based methods have been favoured for the detection of *T. gondii*, however PCR methods lack sensitivity when compared to the bioassay (da Silva and Langoni, 2001; Garcia et al., 2006; Hill et al., 2006). In response, a highly sensitive magnetic capture PCR- method was developed (Opsteegh et al., 2010). The method combines homogenization of a meat sample (100 g) with sequence specific magnetic capture followed by quantitative real time PCR (qPCR) (Opsteegh et al., 2010). Using this method, we aimed to assess the level of *T. gondii* infection in cattle raised and slaughtered in the UK for human consumption. It is expected that the results of this study can inform future probabilistic assessments of the risk of human infection associated with beef consumption.

2. Material and methods

2.1. Study design

A slaughterhouse-based study was conducted in the UK between October 2015 and January 2016. All slaughterhouses across the UK were invited to take part in the study. Ten of them showed willingness to participate and were included. Thus, the selection of slaughterhouses to be included in this study was non-probabilistic and based on voluntary participation. Each slaughterhouse was visited during two or three days, during which one animal was selected for sampling from each farm sending animals to the slaughterhouse during these days. In the case of animals coming in batches brought from livestock markets, the farm where the animal was located before going to market was considered as the farm of origin. The first animal of the batch was sampled, if the first animal of the batch was missed, the second animal was sampled. The target number of animals was 300 for an expected prevalence of 2.9%, 95% confidence interval and 1.9% precision.

The study received ethical approval from the Royal Veterinary College Ethics and Welfare Committee under the reference URN 2015-1407.

2.2. Sample and data collection

A minimum of ~150 g of diaphragm muscle was collected at post mortem from the selected animals. Knives were rinsed and kept in hot water in between diaphragm sampling. Diaphragm samples were placed in polythene bags labelled with a unique ID and sealed to avoid leakage. ID and ear tag numbers from animals sampled were recorded in a standardised recording sheet. Samples were kept and transported on ice and stored at −20 °C until ready for use. Ear tag numbers were used to obtain movement history, age, sex and breed of each animal sampled using the British Cattle Movement System (www.bcms.gov.uk).

2.3. Laboratory analysis

All oligonucleotides used for sequence specific magnetic capture and qPCR were used as designed previously by Opsteegh et al. (2010), targeting the 529-bp DNA fragment (GenBank accession number AF146527). A competitive internal amplification control (CIAC) was included as described by Ospteegh et al. to allow detection of false-negative PCR results (Hoorfar et al., 2004; Opsteegh et al., 2010).

Bovine diaphragm sample preparation and sequence specific magnetic capture was performed as detailed elsewhere (Opsteegh et al., 2010). In between samples, scissors and forceps were rinsed in soap and hot water and then treated with DNAzap (Ambion, Texas, USA) to minimise the risk of cross-contamination. In addition, each filter bag and contents was placed in a second plain Stomacher400 bag during homogenisation to minimise the risk of spillage.

PCR amplification was performed in 96 well plates using the Bio-Rad CFX96 Real time detection system (Bio-Rad laboratories, CA, USA) and SsoAdvanced Universal Probes Supermix (Bio-Rad, CA, USA) in 20 μl reaction volumes per well. Each reaction consisted of 0.7 μM of Tox-9F and Tox-11R, 0.1 μM of Tox-TP1, 0.2 μM of CIAC probe, 0.02 fg of CIAC (kindly provided by M. Opsteegh, as described previously) and 10 μl of template DNA. Cycling conditions were created according to the Supermix manufacturer's recommendations for optimized cycling and comprised of 95 °C for two minutes, followed by 45 cycles 95 °C for 15 s and 60 °C for 30 s. On each plate, a standard series of *T. gondii* DNA was included (ranging from 5×10^7 to 5×10^1). Each reaction was carried out in duplicate and nuclease free water was used in place of DNA in quadruplicate as the non-template (negative) control. The quantification cycle value (Cq) and melt curve was used to determine the *T. gondii* status of all samples. All samples without a Cq value but positive CIAC-PCR were scored negative. Samples with no Cq value for the CIAC-PCR were repeated. Samples with a Cq value for both the *T. gondii* and CIAC assays were scored positive for *Toxoplasma* DNA presence. Samples scored as putative positives were confirmed by standard PCR using magnetically captured DNA as template with primers Tox-9F and Tox-11R (Opsteegh et al., 2010) (Bioline *Taq* polymerase, conditions as described by the manufacturer, 1 × 94 °C for 1 min, 45 x [94 °C for 30 s, 52 °C for 30 s, 72 °C for 30 s], 1 × 72 °C for 7 min). Amplicons were cloned using pGEM-T Easy (Promega) in XL1-Blue MRF *Escherichia coli* (Stratagene), miniprepped (Qiagen) and sequenced (GATC Biotech) as described by the respective manufacturers. Sequence assembly and annotation by BLASTn comparison of homology to the GenBank NR database was undertaken using CLC Main Workbench v6.0.2 (CLC Bio).

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