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Parasite to patient: A quantitative risk model for *Trichinella* spp. in pork and wild boar meat

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ABSTRACT

Consumption of raw or inadequately cooked pork meat may result in trichinellosis, a human disease due to nematodes of the genus *Trichinella*. In many countries worldwide, individual control of pig carcasses at meat inspection is mandatory but incurs high costs in relation to absence of positive carcasses from pigs reared under controlled housing. EU regulation 2015/1375 implements an alternative risk-based approach, in view of absence of positive findings in pigs under controlled housing conditions. Moreover, Codex Alimentarius guidelines for the control of *Trichinella* spp. in meat of suidae have been published (CAC, 2015) and used in conjunction with the OIE terrestrial Animal health code, to provide guidance to governments and industry on risk based control measures to prevent human exposure to *Trichinella* spp. and to facilitate international pork trade.

To further support such a risk-based approach, we model the risk of human trichinellosis due to consumption of meat from infected pigs, raised under non-controlled housing and wild boar, using Quantitative Microbial Risk Assessment (QMRA) methods. Our model quantifies the distribution of *Trichinella* muscle larve (ML) in swine, test sensitivity at carcass control, partitioning of edible pork parts, *Trichinella* ML distribution in edible muscle types, heat inactivation by cooking and portion sizes. The resulting exposure estimate is combined with a dose response model for *Trichinella species* to estimate the incidence of human illness after consumption of infected meat. Paramater estimation is based on experimental and observational datasets.

In Poland, which served as example, we estimated an average incidence of 0.90 (95%CI: 0.00–3.68) trichinellosis cases per million persons per year (Mpy) due to consumption of pork from pigs that were reared under non-controlled housing, and 1.97 (95%CI: 0.82–4.00) cases per Mpy due to consumption of wild boar.

The total estimated incidence of human trichinellosis attributed to pigs from non-controlled housing and wild boar in Poland, is similar to the incidence of human trichinellosis in that country reported by EFSA. Overall, in Europe, we estimated an upper incidence limit of 5.3×10^{-4} cases per Mpy, or less than one predicted case of trichinellosis in the European Union every 4 years, due to consumption of pork from controlled housing. Therefore, *Trichinella* testing of pigs under controlled housing is not adding any value to protect human health. We suggest applying our farm-to-fork QMRA model to further support decision making on the global scale.

1. Introduction

Trichinellosis is a meat borne zoonotic disease in humans caused by nematodes of the genus *Trichinella*. Within this genus, twelve taxa are recognized, nine encapsulated and three non-encapsulated species, which infect a wide range of carnivores and omnivores (Pozio et al., 2009; Pozio and Murrell, 2006; Pozio and Zarlenga, 2013). Domestic pigs, wild boar, and horses are the main animal species through which humans may acquire the infection by consuming contaminated meat.

Infection by *Trichinella* muscle larvae (ML) of humans and other mammalian hosts is followed by maturation of the ingested larvae into adult worms, mating and subsequent release of new born larvae in the small intestine. At least one male and one female larva are required for reproduction, implying that a serving of meat containing one single larva or two larvae of the same sex cannot lead to infection. Newborn larvae penetrate the gut wall and migrate to striated muscle tissues. Clinical disease follows the developmental sequence of *Trichinella*, with varying symptoms depending on the ingested dose and *Trichinella* species (Pozio et al., 2003).

To prevent the disease in humans, domestic pigs and horses, but also wildlife intended for human consumption, such as wild boar, are tested

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for *Trichinella* at slaughter. The parasite preferentially nestles in diaphragm, tongue and masseter in domestic pigs, tongue and masseter in horses and tongue and diaphragm in wild boar (Forbes and Gajadhar, 1999) (Kapel, 2000; Kapel and Gamble, 2000; Kapel et al., 2005). Samples from either of these locations may be taken for testing at slaughter, with preference for diaphragm in domestic pigs, since this is not commercially relevant and is easy to digest, in contrast with tongue. (European-Commission, 2005).

According to Codex Alimentarius, risk management should include a primary production to consumption approach, in order to identify all steps in the food chain where control measures are required (CAC, 2015). Domestic pigs reared in non-controlled housing may become infected under poor hygienic conditions and improper management, involving feeding of non-cooked scraps, offal from slaughter, wildlife remains or ingestion of infected rodents (Oivanen et al., 2002; Pozio, 2001; Pozio et al., 2001a; Schad et al., 1987; Stojcevic et al., 2004). Mandatory requirements for controlled housing in the EU explicitly strive to exclude these risks by maintenance of an efficient rodent control program, acquiring feed from certified producers and storage of feed in closed rodent-proof containers (European-Commission, 2015).

Moreover, the adapted and recently approved EU Regulation 2015/1375 prescribes the method for detecting *Trichinella* ML in muscle tissue (European-Commission, 2015) of individual carcases when controlled housing for pigs is not in place and for all other susceptible animals intended for human consumption, e.g. wild boar. In this method, 100 samples of 1 g diaphragm from domestic pigs or 20 samples of 5 g from wild boar and horse are pooled. Hence, the theoretical test sensitivity is 1 larva per gram is expected for domestic pigs and 1 larva per 20 g for wild boar and horse. However, *Trichinella* spp. ML are not evenly distributed over and within the different muscle tissues of their host (Franssen et al., 2014; Kapel et al., 2005). For this reason, *Trichinella* ML may be missed by random effects at sample collection. To complicate the matter, *Trichinella* counts in diaphragm or other predilection sites differ from each other, and from other muscle tissues, which are used for consumption, such as ham and loin (Kapel et al., 2005).

Trichinella ML that escape detection at meat inspection may be inactivated during household cooking or freezing, but this is prone to failure. Heat inactivation is subject to culinary customs and traditions and undercooked or raw pork or wildlife meat, or raw meat products are frequently consumed. In Eastern Europe, homemade raw salami type sausages made of wild boar meat are traditionally eaten fresh, prior to the two weeks period needed to inactivate *Trichinella* ML larvae in such products (Neghina, 2010; Smith et al., 1989). Meat that is cooked 'rare' or 'medium done', may contain live *Trichinella* ML in its raw core. Some studies have been published concerning heat inactivation of *Trichinella spiralis* larvae through cooking of experimentally *Trichinella* infected pork (Carlin et al., 1969; Kotula et al., 1983) or temperature treatment of encapsulated or naked *T. spiralis* larvae in water (Randazzo et al., 2011).

Risk-based monitoring in the EU has been implemented since 2006 (European-Commission, 2005), which allowed risk-based *Trichinella* testing of domestic pigs in Member States, with derogation from testing for pigs raised in (holdings of) farms under negligible risk, or countries with proven negligible risk. However, since the World Organisation for Animal Health (OIE) no longer recognized a negligible risk status for a country or region, such recognition is linked to compartments of one or more holdings applying specific controlled housing conditions. The negligible risk country status, which was only applied to Denmark and Belgium, was replaced in 2014 by derogation of testing, depending on approval of the controlled housing level of farms or compartments. In parallel, guidelines for risk based control measures of pig meat for global trade have been prepared at the FAO/WHO Codex Alimentarius Committee on Food Hygiene (CCFH) (CAC, 2015). At present, risk-based *Trichinella* monitoring is not supported by a quantitative model to determine the role and impact of measures that restrict the presence of *Trichinella* spp. in pork. Recently, CCFH developed a model to evaluate residual risks of infection with *Trichinella* spp. from *Trichinella*-tested domestic pigs under different hypothetical scenarios (FAO-WHO, 2014). The model in its present form does not include distribution of *Trichinella* numbers over different muscle types and only provides exposure assessment. A dose-response relationship for *Trichinella* infections in humans to translate exposure to human health endpoints is a critical component of quantitative microbial risk assessment (Haas et al., 1999). Other factors relevant to testing for *Trichinella* at meat inspection, such as clustering at animal level, probability of detection or the effect of pooling test samples, are missing as well in the presently available model.

The aim of the present study was to develop a farm-to-fork quantitative microbial risk assessment (QMRA) model that simulates occurrence of the parasite in wildlife, its transmission dynamics through the food chain from meat inspection to consumption of pork or wild boar meat, and consequent human trichinellosis risks. We focus on meat from shoulder, belly and loin, since these meat cuts are purchased raw and cooked by consumers at home. Using the model, we estimate the number of human trichinellosis cases from consuming pork reared in different husbandry systems and from consuming wild boar meat. For this purpose, we evaluated the meat production system in a *Trichinella* endemic country in Europe (Poland), identified critical points at which *Trichinella* ML may escape detection or inactivation, collected and critically appraised relevant datasets to estimate model parameters and developed a stochastic model representing variability due to systematic and random effects. Reported incidence rates of human trichinellosis over a period of six years in Poland are used to evaluate the outcomes of our model. Finally, we discussed uncertainty of model outcome for different parts of our QMRA model.

2. Model

The conceptual model for the *Trichinella* QMRA is shown in Fig. 1, addressing the chain of events between *Trichinella* infection in pigs or wild boar and illness in humans. The next sections describe its modules, for which Table 2 shows the model equations, Table 3 provides the data input and parameters that were used to build the model. All distributions reflect variability. We model numbers of *Trichinella* ML in 100 g of pork originating from fattening pigs from non-controlled housing and wild boar meat, at each step of the food chain to human consumption.

The output of the model is the expected incidence of human trichinellosis in our model country, Poland. We performed 1000 simulations, with each simulation representing a year; therefore, variability over simulations can be interpreted as variability over years. Within each simulation, we model *Trichinella* ML numbers in all portions from 5000 randomly generated carcasses (Fig. 2). All model output was validated by repeated model runs.

2.1. Modules

2.1.1. Trichinella larvae distribution in swine

Data from Polish *Trichinella* control at slaughter in the period 2007–2012 were used to estimate the average *Trichinella* prevalence for wild boar and domestic pigs from non-controlled housing. A negative binomial distribution empirically describes the number of larvae in an animal's diaphragm, also accommodating observed zero larvae for uninfected animals. For parameter estimation, we applied the maximum likelihood method to data sets from Polish surveillance data. These were (1) *Trichinella* ML abundance in 50 g diaphragm samples from thirty-four wild boar (larval loads of 0.3–211 larvae per gram, median 4.92, $n = 34$, Table 1), and (2) the above-mentioned prevalence of *Trichinella* in Polish wild boar.

A number z of *Trichinella* ML is present in the 50 g diaphragm with probability $p(z / m, k)$ (Eq. (1)), where the parameter m is the mean number of *Trichinella* ML in 50 g of diaphragm, averaged over all tested