International Journal of Food Microbiology 241 (2017) 262–275

Contents lists available at ScienceDirect

International Journal of Food Microbiology

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

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

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ARTICLE INFO

Article history:
Received 22 February 2016
Received in revised form 4 September 2016
Accepted 23 October 2016
Available online 26 October 2016

Keywords:
Trichinella
QMRA
Meat inspection
Inactivation
Controlled housing

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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http://dx.doi.org/10.1016/j.ijfoodmicro.2016.10.029

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

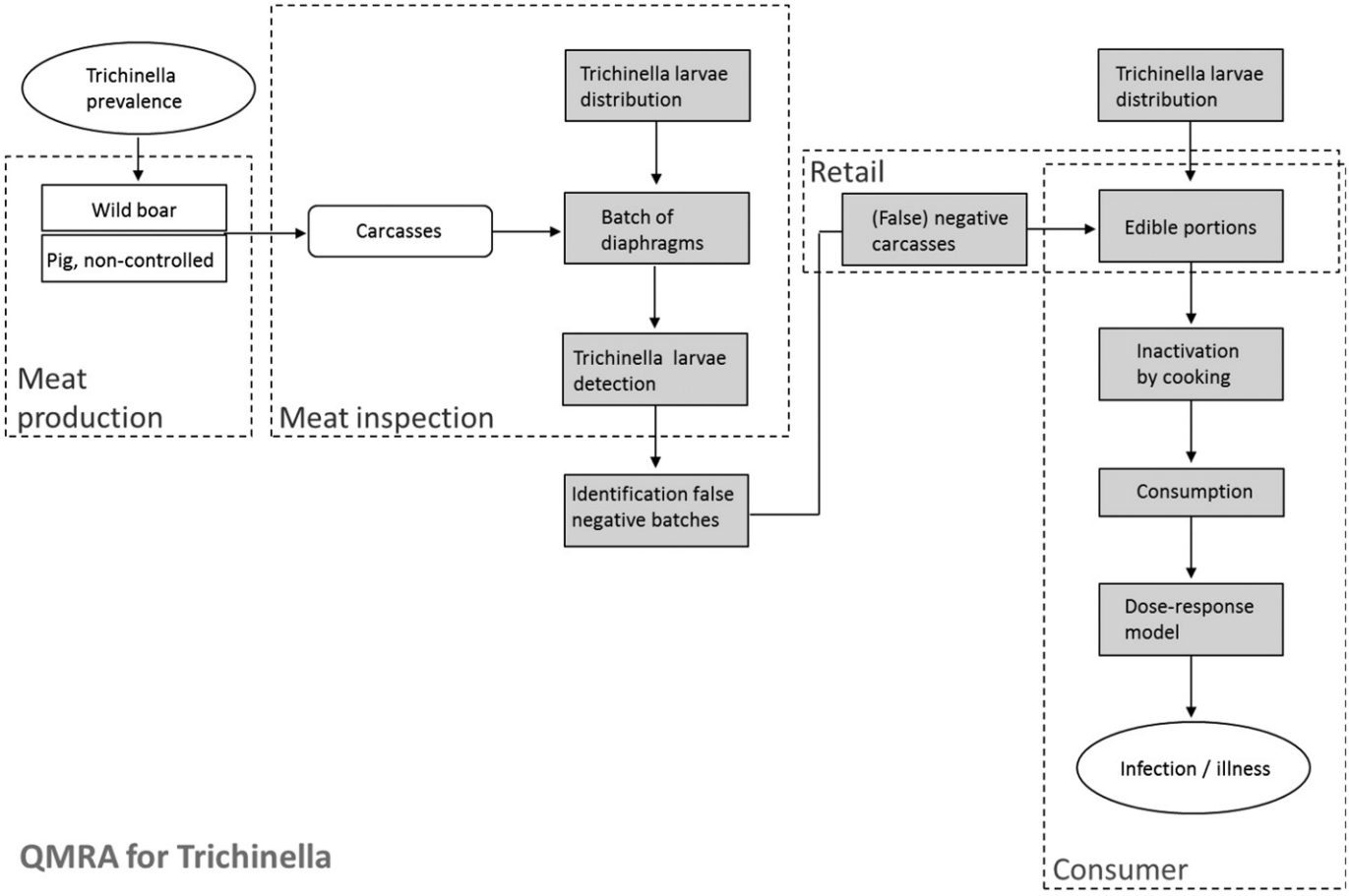

Fig. 1. Conceptual model structure for the quantitative microbial risk analysis for Trichinella in the food chain. Shaded boxes represent modules of the QMRA model.

wild boar, and the parameter k describes the clustering of *Trichinella* ML among individual wild boar. When the value of k is much less than one, the majority of the total larvae in the population of all tested wild boar are present in relatively few animals, while when k approaches infinity, the number of larvae per sample follows a Poisson distribution (no heterogeneity between animals).

Each of the n wild boar tested in the surveillance program was labelled by index j. The likelihood of parasite number z_j is calculated according to Eq. (2) We used $p(0,m,k)$ and $1\text{-}p(0,m,k)$ for the probabilities of parasite absence or presence, respectively. Likelihood of x absent and y present test-outcomes follows a binomial distribution (Eq. (3)). We numerically maximized the log-likelihood to calculate the maximum likelihood estimates for the parameters m and k, using the software Mathematica version 10 (Wolfram Research, Champaign, IL). Now, two 50 g diaphragms make up the total 100 g meat size. The sum of two negative binomial random variables is again a negative binomial distribution with parameters $2\,k$ and $2\,m$.

We also calculated parameters m and k for domestic pigs from non-controlled housing. We substituted for larva-present animals the larval loads determined in wild boar, since count data were not available for domestic pigs from non-controlled housing, and it was anticipated that the distribution of *Trichinella* is comparable in pigs and wild boar, since both are related animals species and potentially exposed to the same environment.

2.1.2. Batch of diaphragm samples for meat inspection

According to EU legislation, for wild boar, the *Trichinella* test procedure at meat inspection (artificial digestion) utilises 5 g of diaphragm muscle of 20 wild boars each, in a pool of in total 100 g muscle. For pigs, 1 g of diaphragm muscle is used, and samples from 100 pigs are pooled. In this study, we simulated 600,000 wild boar or 114×10^6 pigs from non-controlled housing being tested during meat inspection at slaughter in a simulated year. These numbers represent the average slaughter volumes in Poland in 2007–2012 (EFSA-ECDC, 2010, 2013, 2014).

We label each swine with an index j and sample for each animal according to the negative binomial distribution with parameters $2\,m$ and $2\,k$ a number Y_j of *Trichinella* ML in 100 g of diaphragm (Eq. (4)).

Testing the diaphragm of each animal involves determining a subset of *Trichinella* ML that are present in 5 g diaphragm sample of wild boar or 1 g diaphragm sample for pig. We chose to model sampling from 100 g of diaphragm, which is the standardised portion size throughout the model, instead of modelling sampling from 200 g of diaphragm (estimated total weight) and subsequent downscaling. Sampling is a binomial process with Y_j trials and probability of $p = 0.05 = 5\text{ g} / 100\text{ g}$ for wild boar and $p = 0.01 = 1\text{ g} / 100\text{ g}$ for pigs for each larva to be present in a fraction of the diaphragm weighing 5 g and 1 g, respectively. A random draw y_q from the binomial distribution with parameters p and Y_j determines the number of larvae in 5 or 1 g diaphragm for each wild boar or domestic pig, respectively. Add up each realisation per animal in the pool, to arrive at the total number R of larva in the 100 g pool (Eq. (5)). We keep track of which animal contributes to which pool, to trace individual animals in a false negative batch later on.

2.1.3. Trichinella muscle larvae detection

We model *Trichinella* ML detection at meat inspection by quantifying the probability of detecting one single larva in the pool. For this, we used 280 proficiency test records of the Dutch National Reference Laboratory

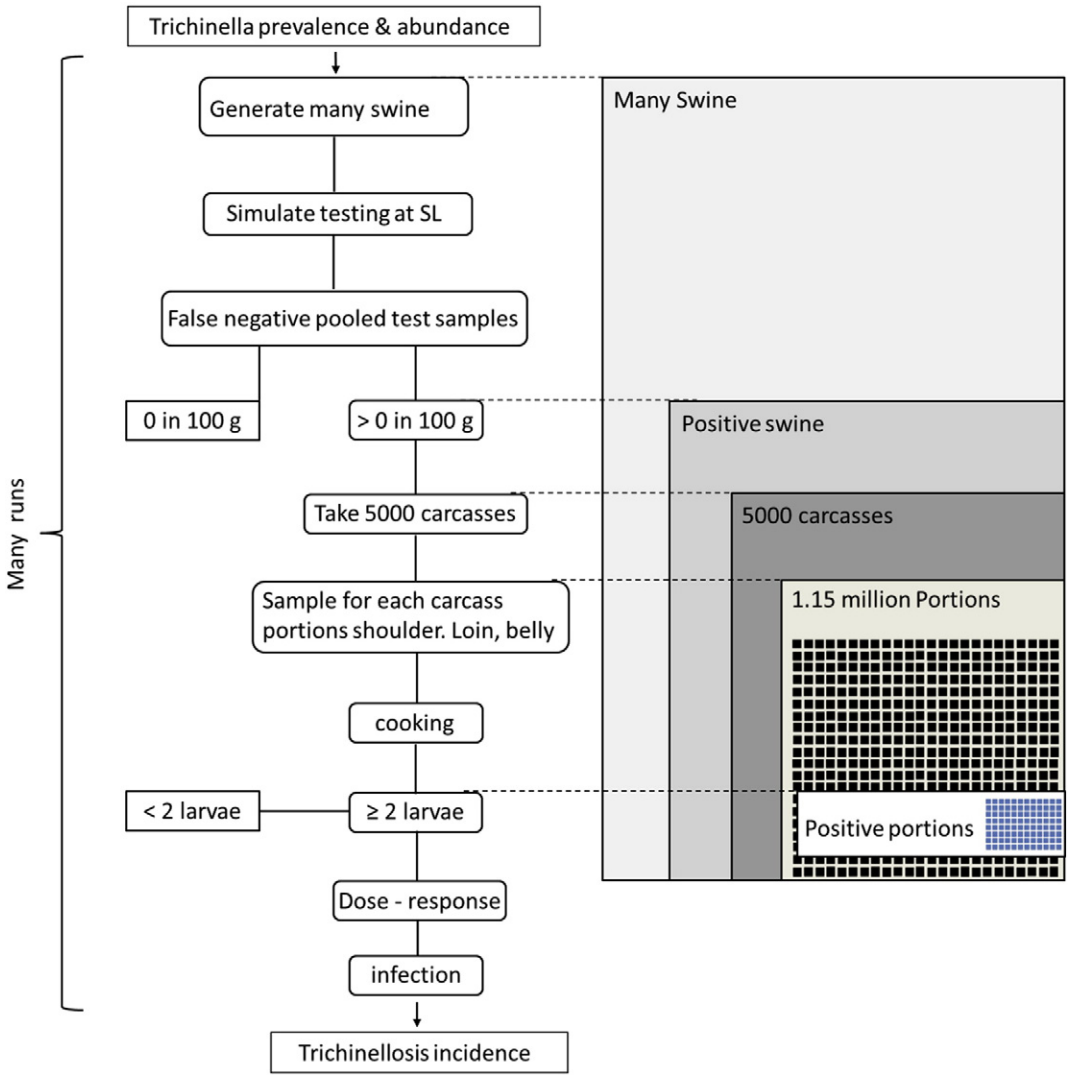

Fig. 2. Model layout of the QMRA for Trichinella. From top to bottom, the boxes indicate the model flow as corresponding to Fig. 1. Branching steps are indicated. The right hand side gives a visual representation of the relative number of modelled units (i.e after testing of pooled samples the model unit is 'positive swine', there are less of those than 'swine').

for Parasites (RIVM, Bilthoven, the Netherlands), in which a known number of *Trichinella* ML were added to 100 g of muscle tissue and 14 technicians from four different routine *Trichinella* laboratories counted the recovered *Trichinella* ML (Table 4). Recovery of each larva is a random process, with the probability of recovery for each larva unlikely to be the same for all larvae because different technicians and

Table 1
Trichinella larval burden in 34 individual Polish wild boar.

ID	Province	Species	LPG	ID	Province	Species	LPG
20	Warmińsko - Mazurskie	*T. britovi*	7.6	467	Mazowiecie	*T. spiralis*	0.22
55	Lubuskie	*T. spiralis*	4.02	474	Podlaskie	*T. britovi*	89
59	Zachodnio - Pomorskie	*T. britovi*	0.3	481	Zachodnio - Pomorskie	*T. spiralis*	19.52
61	Kujawsko - Pomorskie	*T. spiralis*	42.8	485	Świętokrzyskie	*T. britovi*	47
137	Podlaskie	*T. britovi*	33.1	513	Pomorskie	*T. britovi*	2.4
143	Warmińsko - Mazurskie	*T. britovi*	12	519	Warmińsko - Mazurskie	*T. britovi*	3
146	Zachodnio - Pomorskie	*T. britovi*	13	521	Zachodnio - Pomorskie	*T. spiralis*	4.1
203	Warmińsko - Mazurskie	*T. spiralis*	0.3	539	Zachodnio - Pomorskie	*T. spiralis*	69
320	Kujawsko - Pomorskie	*T. spiralis*	41	579	Lubelskie	*T. spiralis*	4.92
322	Zachodnio - Pomorskie	*T. spiralis*	1.9	594	Zachodnio - Pomorskie	*T. spiralis*	56
326	Mazowieckie	*T. spiralis*	4	597	Kujawsko - Pomorskie	*T. spiralis*	211
378	Lubuskie	*T. spiralis*	10	609	Zachodnio - Pomorskie	*Trichinella* sp	0.8
415	Pomorskie	*T. spiralis*	2.4	610	Mazowiecie	*T. spiralis*	0.9
429	Warmińsko - Mazurskie	*T. britovi*	1.4	622	Wielkopolskie	*T. spiralis*	94
432	Warmińsko o Mazurskie	*T. britovi*	6	624	Wielkopolskie	*T. spiralis*	0.56
445	Wielkopolskie	*T. spiralis*	63	630	Lubelskie	*T. britovi*	2.12
458	Zachodnio - Pomorskie	*T. britovi*	4.92	652	Opolskie	*T. spiralis*	1.5

ID: identification of individual wild boar; LPG: larvae per gram.

Table 2

Model step	Symbol	Equation	Equation
2.1.1 *Trichinella* larvae distribution in swine	p: probability density function z: number of *Trichinella* muscle larvae (ML) m: mean number of *Trichinella* ML in 50 g k: a measure of unevenness in *Trichinella* ML counts among wild boars	$p(z\|m,k) = \frac{(k+z-1)!}{z!(k-1)!}(1+\frac{m}{k})^{-k-z}(\frac{m}{k})^{z}$	1
	L: likelihood n: number of swine observed j: index of individual swine z_j: number of *Trichinella* ML in individual swine	$L(m,k\|z_j) = \prod_{j=1}^{n} p(z_j,m,k)$	2
	B: likelihood x: number of swine with no *Trichinella* ML present y: number of swine with *Trichinella* ML present	$B(x,y\|m,k) = \binom{x+y}{y}(1-p(0,m,k))^{y}p(0,m,k)^{x}$	3
2.1.2 Batch of diaphragm samples for meat inspection	p, z, m, k as in 2.1.1	$p(z\|2m,2k) = \frac{(2k+z-1)!}{z!(2k-1)!}(1+\frac{m}{k})^{-2k-z}(\frac{m}{k})^{z}$	4
	Q: number of animals in a pool y_q: number of *Trichinella* ML in a diaphragm sample from an individual animal R: number of *Trichinella* ML in a pool	$R = \sum_{q=1}^{Q} y_q$	5
2.1.3 Trichinella larvae detection	g: probability density function U: number of *Trichinella* ML per test sample	$g(U,u) = \binom{U}{u}\frac{Beta(u+a,U-u+b)}{Beta(a,b)}$	6
	u: *Trichinella* ML recovered by technician Beta: Euler beta function a: a shape parameter of beta distribution b: parameters of the beta function	$Beta(a,b) = \int_0^1 t^{a-1}(1-t)^{b-1}dt$	7
2.1.5 Number of edible portions and *Trichinella* ML distribution	n_E: number of edible pork portions of muscle group m w: weight of muscle group m in kg m_{fract}: lean mass fraction of muscle group m	$n_E = (w \times m_{fract})/0.100$	8
	C: vector of *Trichinella* ML counts in muscle parts M: number of distinct muscle parts p_i: see Table III line 2.1.5 Y_j: observed number of *Trichinella* ML in muscle part $M+1$ (diaphragm)	$P(C=c) = \binom{Y_j+M-1}{M}\binom{M}{c_1 \cdots c_M}\prod_{i=1}^{M} p_i^{c_i} p_{M+1}^{Y_j}$ $C = (C_1, \ldots, C_M)$ $c = (c_1, \ldots, c_M)$ l_i is a random draw from C	9
	f: probability density function l_i: number of *Trichinella* ML to distribute for muscle part i v: vector of *Trichinella* ML counts over portions	$f(v,l) = \frac{l_i!}{\prod_{k=1}^{n_E} x_k!} 2^{-\sum_{k=1}^{n_E} x_k}$ h_i *is a random draw from* f	10
2.1.6 Inactivation by cooking	I_1: remaining number of *Trichinella* ML after inactivation at time point t_1 h_i: starting number of *Trichinella* ML after inactivation at time point t_0 α^+: maximum inactivation possible K: steepness at point T^* T^*: point of maximal slope T_0: lower temperature T_1: upper temperature t_1: time point at which T_1 is reached	$\log(I_1) = \log(h_i) + \frac{\alpha^+ t_1}{k(T_0-T_1)} \log(\frac{1+e^{k(T_1-T^*)}}{1+e^{k(T_0-T^*)}})$ Note that this equation is applied several times, for different phases in the cooking process. We do not repeat the formula for each step for clarity.	11
2.1.7 Consumption patterns	A_{Wild}: available number of consumable portions per year N_{wild}: total number of wild boar $\sum p$: total number of portions shoulder, loin and belly per carcass PL_C: total population in Poland (period 2007 – 2012)	$A_{Wild} = N_{wild} \times \sum p / PL_c$	12
	N_{con}: number of consumed portions of pork per year in Poland P_{nc}: proportion of non-controlled housing in Poland C_{Pork}: average number of consumed portions of pork shoulder, loin and belly (100g) for Poland per year PL_A: average population in Poland (period 2007 – 2012)	$N_{con} = P_{nc} \times C_{Pork} \times PL_A$	13
2.1.8 Dose response modelling	P_{ill}: probability of illness I_1: number of *Trichinella* ML after cooking P_m: *Trichinella* ML survival probability r: sex ratio (e.g. proportion of females)	$P_{\mathrm{ill}}(I_1\|P_m,\mathrm{r}) = 1 + e^{-I_1 pm} - e^{-I_1 pm(1-r)} - e^{-I_1 pmr}$ $P_m \sim \mathrm{Beta}(\alpha,\beta)$	14
2.2 Risk characterisation	$P_{ill,Wild}$: total predicted human cases per year from consumption of wild boar meat A_{Wild}: available number of consumable portions per year (see 2.1.7) $N_{ill\ Wild}$: average illness per million consumable portions (see 2.1.7)	$P_{Wild} = A_{Wild} \times N_{ill,Wild}$	15
	N_{con}: number of consumed portions of pork per year (see 2.1.7) P_{nc}: proportion of non-controlled housing in Poland (see 2.1.7) C_{Pork}: average number of consumed portions of pork (100g) for Poland per year (see 2.1.7) $N_{ill,pork}$: average number of illness per million portions of pork from non-controlled housing $P_{ill,pork}$: total predicted human cases per year from consumption of pork from non-controlled housing	$P_{ill,pork} = N_{con} \times N_{ill,pork}$	16
2.3 Extrapolation to pigs from controlled housing	g: probability density function n: number of swine for testing u: possible number of positive swine A: number of positive swine in past tests B: number of swine tested in past tests U_p: upper prevalence limit	$g(n,\mathrm{u}) = \binom{n}{u}\frac{Beta(u+A+1,n-u+B+1)}{Beta(A+1,B+1)}$	17
	T_{EU}: number of annually tested pigs from controlled housing T_p: testing period	$U_P = (T_{EU} \times T_p)^{-1}$	18
3.3.5 Extrapolation to pigs	$P_{ill,extra}$: maximum extrapolated number of human trichinellosis cases	$P_{ill,extra} = U_P \times p^{-1} \times P_{ill,pork}$	19

Table 2 (*continued*)

Model step	Symbol	Equation	Equation
from controlled housing	per year from consumption of pork from controlled housing U_P: upper limit in prevalence for controlled housing p = prevalence in pigs from non-controlled housing $P_{ill,pork}$: total predicted human cases per year from consumption of pork from non-controlled housing (see 2.2) $P_{ill,extra}$: maximum extrapolated number of human trichinellosis cases per year from consumption of pork from controlled housing F_{ill}: occurrence of $P_{ill,extra}$ *in years*	$F_{ill} = (P_{ill,extra} \times 508)^{-1}$	20

Table 3
Data inputs and parameters in the model.

Model part	Symbol	Unit	Wild boar	Pig non-controlled	Reference
2.1.1 *Trichinella* larvae distribution in swine	m: mean number of *Trichinella* ML in 50 g	Larvae/50 g	5.24	0.00159125	This study
	k: larvae distribution between swine	–	4.47×10^{-4}	1.35×10^{-7}	This study
	n: number of swine tested	Swine	685,595	114,395,817	EFSA-ECDC, 2010, 2013, 2014
	z_j: number of *Trichinella* ML in individual swine	*Trichinella* ML/animal	0.3–211	0.3–211	Observed (Table 1)
	x: number of *Trichinella* ML -absent swine	Swine	682,763	114,395,672	EFSA-ECDC, 2010, 2013, 2014
	y: number of *Trichinella* ML -present swine	Swine	2832	145	EFSA-ECDC, 2010, 2013, 2014
2.1.2 Probability for a Trichinella ML to be present in 5 or 1 g of diaphragm	p: probability for a *Trichinella* ML to be present in G grams of diaphragm	–	0.05	0.01	This study
	Q: number of animals per pool	Swine	20	100	EU 2075/2005
2.1.3 *Trichinella* larvae detection	U: number of *Trichinella* ML per test sample	*Trichinella* ML/100 g	0–9 (median = 5)	0–9 (median = 5)	Experimental data
	u: *Trichinella* ML recovered by technician	*Trichinella* ML	0–10 (median = 4)	0–10 (median = 4)	NRL-P (NL)
	a: shape parameter	–	1.54	1.54	This study
	b: shape parameter	–	0.29	0.29	This study
2.1.4 Identification of false-negative batches	G: weight of diaphragm sample	g	5 g	1 g	EU 2075/2005
	Q: number of animals per pool	Swine	20	100	EU 2075/2005
2.1.5 Number of edible portions and *Trichinella* ML distribution	n_E: number of edible pork portions	Portions	367	395	Estimated (Table 4)
	w: weight of muscle group m in kg	kg	8.00–21.03 kg	7.86–29.28 kg	Estimated (Table 4)
	m_{fract}: lean mass fraction of muscle group m*	Percent	57%	44–62%	Marcoux et al., 2007; Monziols et al., 2006; Skewes et al., 2008
	n_p: number of portions calculated from weight (shoulder, belly, loin)	Portions	50, 76, 103	35, 54, 144	This study (Table 4)
	P_i: probability of allocation to muscle part (shoulder, belly, loin)	–	0.103, 0.278, 0.101	0.059, 0.162, 0.116	This study (Table 7)
2.1.6 Inactivation by cooking	T^* point of maximal slope	°C	59.3	59.3	Swart & Franssen, in preparation
	K: steepness at point T^*	–	0.17	0.17	
	α^+: maximum inactivation possible	–	0.63	0.63	
2.1.7 Consumption patterns	A_{wild}: available number of 100 g portions of wild boar meat	Portion/person/year	0.68		This study
	N_{wild}: total number of tested wild boar (period 2007–2012)	Animals	685,595	–	EFSA-ECDC, 2010, 2013, 2014
	$\sum p$: total number of portions shoulder, loin and belly per carcass	Portions	229	223	Estimated (Table 6)
	PL_C: total population in Poland (period 2007–2012)	Humans	230,020,458	230,020,458	EFSA-ECDC, 2010, 2013, 2014
	Ncon: number of consumed portions of pork per year in Poland	Portions	2.71×10^7	1.54×10^{10}	This study
	P_{nc}: proportion of non-controlled housing in Poland	Proportion	–	0.784	(Pozio, 2014)
	C_{Pork}: average number of consumed portions (100 g) for Poland per year	Portions	0.68	314	This study
	PL_A: average population in Poland (period 2007–2012)	Persons	3.83×10^7	3.83×10^7	This study
2.1.8 Dose response modelling	r: sex ratio (e.g. proportion of females)	Proportion	0.70	0.70	Teunis, 2012
	α, β: dose response parameters		sampled from uncertainty distribution	sampled from uncertainty distribution	Supplied by Teunis
2.3 Extrapolation to pigs from controlled housing	A: number of positive swine in past tests	–	0	0	EFSA-ECDC, 2010, 2013, 2014
	B: number of swine tested in past tests	Pigs	–	139,729,393	EFSA-ECDC, 2010, 2013, 2014

* Only average value without variation given for wild boar in Skewes et al., 2008.

Table 4
Results of proficiency tests organised by the Dutch NRL-P during the period 2012–2014. Data represent Trichinella muscle larvae that were spiked in 100 g fat-free minced pork balls, which were recovered by different technicians at slaughterhouse labs after artificial digestion according to EU regulation 2075/2005. Rec: recovered.

Spike	Rec	Spike	Rec	Spike	Rec	Spike	Rec	Spike	Rec	Spike	Rec	Spike	Rec
5	5	5	4	3	3	3	3	5	5	4	4	8	6
5	5	0	0	3	3	0	0	0	0	8	6	0	0
7	7	5	3	8	7	3	1	5	4	0	0	4	4
0	0	7	6	0	0	8	3	9	6	6	6	8	7
9	4	9	9	8	6	8	6	9	6	8	8	6	5
5	5	5	4	3	4	3	3	9	7	4	5	6	6
5	4	0	0	3	3	0	0	5	4	8	8	8	8
7	7	5	3	8	7	3	1	0	0	0	0	0	0
0	0	7	4	0	0	8	3	9	9	6	6	8	8
9	4	9	8	8	8	8	6	5	4	8	8	4	4
5	5	7	5	3	3	3	3	9	6	4	2	6	5
5	4	9	8	3	3	0	0	5	5	8	5	8	6
7	7	5	4	8	7	3	1	0	0	0	0	0	0
0	0	0	0	0	0	8	3	9	8	6	6	8	7
9	6	5	3	8	7	8	5	5	6	8	7	4	4
5	4	7	5	3	3	3	3	9	7	4	3	6	4
5	4	9	6	3	3	0	0	5	4	8	6	8	6
7	7	5	4	8	7	3	1	0	0	0	0	0	0
0	0	0	0	0	0	8	3	9	9	6	6	8	6
9	4	5	4	8	8	8	6	5	4	8	8	4	4
9	8	7	4	3	3	3	3	5	5	4	4	8	6
5	5	9	8	0	0	0	0	0	0	8	6	0	0
0	0	5	3	3	2	3	1	9	8	0	0	4	4
5	5	0	0	8	6	8	3	5	3	6	6	6	5
7	6	5	2	8	5	8	6	9	8	8	8	8	4
9	7	3	3	3	2	5	5	5	6	8	7	8	5
5	6	3	2	0	0	0	0	0	0	0	0	0	0
0	0	8	7	3	2	5	4	9	8	4	3	4	3
5	4	0	0	8	6	9	6	5	3	8	8	6	3
7	7	8	6	8	5	9	7	9	8	6	6	8	6
9	8	3	3	3	3	5	4	5	5	8	5	8	6
5	5	3	3	0	0	0	0	0	0	0	0	0	0
0	0	8	7	3	2	5	3	9	7	4	4	4	2
5	4	0	0	8	7	9	8	5	3	8	8	6	3
7	6	8	6	8	5	9	7	9	8	6	6	8	5
5	4	3	3	3	3	5	5	5	5	8	5	8	7
0	0	3	2	0	0	0	0	0	0	0	0	0	0
5	3	8	7	3	1	5	3	9	10	4	4	4	3
7	5	0	0	8	3	9	6	5	3	8	7	6	4
9	9	8	6	8	5	9	7	9	8	6	6	8	3

laboratories are involved in such analyses. We describe the variability in the recovery probability by a Beta binomial distribution $g(U,u)$ (Eq. (6)), which is a mixture of binomial and beta distributions.

In a testing meat sample to which U larvae were added, a technician recovered a number of u larvae following a Beta distribution with parameters a and b, which is written in terms of the Euler beta function (Eq. (7)).

Using the software Mathematica (Wolfram Research, Champaign, IL) the log-likelihood was numerically maximized to calculate the maximum likelihood estimates for the parameters a and b (2.1.3., Table 3).

2.1.4. Identification of false-negative batches

All carcasses that correspond to a pool that tests positive for *Trichinella* ML at meat inspection are withdrawn from the food chain and therefore do not constitute a risk to consumers. However, a batch containing $R > 0$ larvae may pass the meat inspection, i.e. *Trichinella* ML are not detected, with a probability $g(R,0)$. If this happens, we mark the batch as a false-negative.

After identification of the false-negative batches, we trace back the individual infected swine(s) in this batch. For each of these batches, we list the number Y_j of *Trichinella* ML present in the 100 g diaphragm(s) of the individual animal(s).

2.1.5. Number of edible portions and Trichinella ML distribution

The output generated in the previous paragraph needs three further steps to identify the number of *Trichinella* ML for each consumable portion of pork or wild boar meat.

Step 1 is to determine the number of portions that are available for consumption from relevant muscle parts. The average weight of commercially relevant swine muscle parts was determined using published experimental data of several pig breeds and of farmed wild boar (Marcoux et al., 2007; Monziols et al., 2006; Skewes et al., 2008) (Table 6). Commercial pork cuts are shoulder, foreleg, loin, rib, belly and ham, but the most relevant pork parts were considered to be loin, shoulder and belly (including intercostal and abdominal muscles), since the consumer purchases these parts raw and food safety relies on proper home cooking procedures. Foreleg is generally eaten well cooked and thus we excluded this type of meat. For each selected pork part, we calculated the number of edible 100 g portions (Eq. (8)).

In step 2, we estimated the number of *Trichinella* ML in edible muscle parts from the number of *Trichinella* ML in 100 g of diaphragm. Experimental infection data obtained over eight years, using one domestic pig per year, revealed the distribution of *Trichinella* ML in 100 g of diaphragm, tongue, masseter, shoulder, foreleg, abdominal and intercostal muscles (=belly), loin and ham of each animal (Table 6).

Given the numbers present, the distribution of the larvae in the muscle groups is assumed multinomial, but unlike the common situation where one knows the total, and is interested in counts in categories, we now know counts in a single category (diaphragm) and we wish to estimate the counts in the other categories. For this purpose the negative multinomial distribution is suitable ((Lange, 2010) page 139), where C is a vector of larvae counts, and the probability distribution describes the probability of finding $C_1 = c_1, \ldots, C_M = c_M$ larvae (Eq. (9)). The number of muscle parts is $M + 1$, with category $M + 1$ being the

Table 5
Muscle weight distribution of pork cuts. (adapted from Monziols et al., 2006 and Skewes et al., 2008). Lean Muscle: lean mass fraction of muscle group.

Domestic pig	Weight (kg)	Lean muscle	Muscle (kg)	# Portions
Live weight	115			
Carcass	81.8	0.50	40.6	406
Shoulder[2]	20.7	0.45	9.3	93
Shoulder[3]	7.9	0.45	3.5	35
Foreleg[3]	8.8	0.45	3.9	40
Belly[4]	12.4	0.44	5.4	54
Loin[5]	29.3	0.49	14.4	144
Ham	19.6	0.62	12.1	121
Total			39.5	395
Wild boar	Weight (kg)	Lean muscle	Muscle (kg)	# Portions
Carcass[1]	81.8	0.57	46.6	466
Shoulder[2]	20.0	0.57	11.4	114
Shoulder[3]	8.8	0.57	5.0	50
Foreleg[3]	3.2	0.57	1.8	18
Belly[4]	13.3	0.57	7.6	76
Neck loin[3]	8.0	0.57	4.6	46
Loin	9.9	0.57	5.7	57
Loin[5]	17.9	0.57	10.3	103
Ham	21.0	0.57	12.0	120
Total:			36. 7	367

Domestic pigs.
Average of 24 pigs, 6 each of four different breeds. [2]Shoulder in Canadian cuts includes shoulder, neck and foreleg of a pig's carcass. [3]% of carcass weight (Marcoux et al., 2007) was used to separate shoulder and foreleg (European cut) from Schoulder[2] (Canadian cut). [4]Belly includes intercostal and abdominal muscles. [5]neck loin and loin are considered jointly, since they will probably be prepared similarly by consumers.
Wild boar
[1]Carcass weight given in the paper was 47.2 kg for farmed wild boar of 39 weeks old; this weight was standardised to the average weight for domestic pigs, which would represent the weight of an average adult wild boar (live weight 75–160 kg, depending on age and gender). [2]Chilean pork cut is identical to Canadian cut. [3]These separate parts were given in the paper by Skewes et al. [4]Pork cut 'Belly' is called 'Spare rib' in Chilean cut. [5]Neck loin and loin are considered jointly, since they will probably be prepared similarly by consumers.

diaphragm for which the number of larvae Y_j is known in the model. The probabilities p_i are estimated in the usual way, by dividing the number of larvae in category i by the total (see columns 'Fraction' Table 6).

In step 3, the number of *Trichinella* ML in each muscle part was assigned to the portions by using a multinomial distribution with equal probability for each portion (Eq. (10)). This output entered the next part of the model.

2.1.6. Inactivation

2.1.6.1. Cooking. The risk of contracting trichinellosis is related to the number of eating occasions and portion size, the number of false negative pools that escape detection at meat inspection, the prevalence of positive carcasses in those pools, the number of larvae per gram of edible meat, *Trichinella* ML that escape inactivation by cooking and the sex distribution of *Trichinella* ML.

To evaluate heat-inactivation of *Trichinella* ML in meat, we extracted data from the limited available literature on *Trichinella* ML inactivation by oven cooking of pork from experimentally infected pigs (Carlin et al., 1969) and cooking of naked larvae in test tubes containing water (Randazzo et al., 2011). We modelled *Trichinella* ML inactivation as a function of temperature and time. The model for the probability of *Trichinella* ML inactivation as a function of temperature at a given cooking time, is approximated by an ascending sigmoidal curve. T^* is the point where the temperature inactivation curve reaches its maximal slope. Eq. (11) describes residual numbers of larvae (I_1) after inactivation of a portion of meat containing I_0 larvae at time point $t_0 = 0$ and temperature T_0, following heating to temperature T_1 at time point t_1. (Swart and Franssen, manuscript in preparation).

Finding a realistic cooking scenario in literature is difficult and published experiments seldom fit ones purpose exactly. For the model, we used a set of cooking scenarios, which included an experimental temperature-time profile for the preparation of pan-fried patties weighing 115 g (dimensions: 2,5 cm thick, 8.5 cm in diameter) (Swart et al., 2015). Furthermore, we used USDA temperature recommendations for consumer pork cooking (USDA, 2015), and results of a small scale questionnaire, which revealed that 15% of responders cooked pork chops medium done (scenario 'Medium Swart') and 85% cooked pork chops well done (scenario 'Chef') (http://donetemperature.com/) or 'Traditional' (well done plus extra cooking time, Table 7).

Cooking preferences are personal and diverse, often passed on between generations and difficult to capture. Consumer preference data from UK (Prior et al., 2014) and Dutch reports (Swart et al., 2015) showed that 8–12% of the population eats meat 'that has pink or red

Table 7
Different cooking scenarios for pork and wild boar meat.

	Scenario settings			
Scenario	T_0 (°C)	T_1 (°C)	t_1 (min)	Extra (min)
Uncooked	–	–	–	–
Rare, Swart	20	54	2.5	–
Medium Swart	54	63	4.2	–
Well done Swart	54	68	5.3	2.0[a]
Medium, USDA	54	63	4	3.0[b]
Well done, USDA	54	71	9	–
Well done, Chef	54	76.7	10	–
Well done, Traditional	54	76.7	10	10.0[c]

The first stage of every scenario is 'Rare, Swart', e.g. scenario Medium Swart utilises 2.5 min to reach 54 °C, followed by 1.7 min continued cooking to reach a final temperature of 63 °C, and a final cooking time of 4.2 min. See Fig. 3 for an overview of cooking scenario profiles.
[a] Additional cooking time at 68 °C.
[b] Resting time at 63 °C.
[c] Additional cooking time at 76.7 °C.

Table 6
Number of Trichinella ML detected in different muscle types of experimentally infected pigs, and estimated distribution of ML over 100 g portions of edible tissue from 1 pig.

Year	2005	2006	2007	2008	2009	2010	2012	2013							
Infection dose	37,000	40,000	40,000	20,000	40,000	40,000	40,000	40,000		Wild boar			Domestic pig		
Incubation time (weeks)	23	19	19	26	16	10	13	16	Row sums	Portions	Total ML	Fraction	Portions	Total ML	Fraction
Diaphragm pillar	460	469	645	288	1148	239	1332	821	5402	2[a]	10,804	0.0134	2[a]	10,804	0.0110
Shoulder	94	232	194	102	297	107.5	396	230	1652.5	50	82,625	0.1028	35	57,837	0.0590
Belly	303	214	272	146	658	155.5	725	463	2936.5	76	223,174	0.2776	54	158,571	0.1618
Loin	72	79	59	44	132	40.5	206	156	788.5	103	81,215	0.1010	144	113,544	0.1159
Ham	80	93	110	76	217	53	309	161	1099	120	131,880	0.1640	121	132,979	0.1357
Other[b]	1225	1112	1322	752	1895	559	2199	1480	10,544	26	274,144	0.3410	48	506,112	0.5165
Column sums	2234	2199	2602	1408	4347	1154.5	5167	3311	22,422.5		803,842	1		979,847	1

Total numbers of Trichinella ML (Total ML) were determined by multiplication of Row sums with number of portions as described in Table 5. From these totals, fractions were calculated, spanning eight independent experimental infections.
[a] The weight of diaphragm was estimated at 200 g.
[b] 'Other' represents tongue, masseter and foreleg; the weights of tongue and masseter were estimated at 400 g each, the weight of foreleg is shown in Table 4. Data adapted from (Mayer-Scholl et al., 2012; Mayer-Scholl et al., 2009, 2010; Mayer-Scholl et al., 2011; Nöckler and Reckinger, 2005).

Table 8
Model outcomes calculated for three swine production systems: hunted wild boar, pigs from non-controlled housing and pigs from controlled housing.
A. Probability that a swine is from a false negative batch. B. Prevalence of positive carcasses from false negative batches; C. Prevalence of positive portions before cooking, over all portions of positive carcasses; D. Prevalence of positive portions after cooking, over all portions of positive carcasses; E.Probability of illness, for each of the portions from positive carcasses; F. Number of illnesses per million portions of 100 g. G. Average number of illnesses per million portions of any muscle type.

	Wild boar		Pig, non-controlled housing	
Carcass testing	Mean	95%CI	Mean	95%CI
A. False negative pools	3.63×10^{-3}	2.17×10^{-3}–4.90×10^{-3}	6.03×10^{-6}	0.00–1.65×10^{-5}
B. Positive carcasses in those pools	5.32×10^{-2}	5.00×10^{-2}–5.93×10^{-2}	0.010	0.010–0.010
C. Trichinella infected portions from positive carcasses				
Shoulder	0.912	0.821–0.986	0.739	0.000–1.000
Belly	0.716	0.576–0.869	0.731	0.000–1.000
Loin	0.751	0.615–0.890	0.697	0.000–1.000
D. Residual infected cooked portions from positive carcasses				
Shoulder	0.273	0.155–0.408	0.536	0.000–1.000
Belly	0.080	0.048–0.148	0.411	0.000–1.000
Loin	0.090	0.053–0.156	0.240	0.000–0.990
E. P illness from cooked portions from positive carcasses				
Shoulder	0.029	0.016–0.050	0.081	0.000–0.248
Belly	0.007	0.003–0.017	0.046	0.000–0.162
Loin	0.009	0.004–0.020	0.021	0.000–0.084
F. N illness per million portions				
Shoulder	5.55	2.440–10.0	0.006	0.000–0.021
Belly	1.41	0.541–3.41	0.003	0.000–0.013
Loin	1.71	0.655–3.95	0.001	0.000–0.006
G. Average N illness per million portions	2.89	1.21–5.88	0.004	0.000–0.013
H. N consumptions of 100 g pp/year	–	6.80×10^{-1}	–	246[a]
I. Average population size 2007–2012	–	3.83×10^{7}	–	3.83×10^{7}
J. Total consumed portions	–	2.61×10^{7}	–	1.54×10^{10}
K. Total predicted human cases/year	75.3	31.6–153	34.6	0.00–141
L. Predicted human cases/million/year	1.97	0.82–4.00	0.904	0.00–3.68

[a] The average pork consumption per capita per year is 514 portions of which 58.99% of portions originate from shoulder, loin and belly (=314 portions); the proportion of origin from non-controlled housing is estimated at 78.4% (Pozio, 2014), which equates 246 portions.

juices', indicating medium or rare cooked meats, including pork and sausages. The frequency at which this happened was reported as 'some times' to 'regularly'. For the model, we assumed that 10% of the general population eats risky meat, defined as pork that has been cooked to a core temperature of no >63 °C (scenario 'Medium, USDA') and 90% of the population cooks pork to 'Well done Chef'. All scenarios include scenario 'Rare, Swart' as first stage, after which each scenario continues with its own profile (Fig. 3).

Table 9
Analysis of trichinellosis incidence following different cooking scenarios.

Wild boar	Trichinellosis cases/year			Cases/million persons/year		
Scenario	Average	2.5 perc	97.5 perc	Average	2.5 perc	97.5 perc
Uncooked	747	430	1120	19.5	11.2	29.2
Rare, Swart	713	405	1090	18.6	10.6	28.3
Medium Swart	511	268	810	13.3	6.98	21.1
Well done Swart	220	96.7	574	5.74	2.52	10.3
Medium, USDA	190	85.2	34.2	4.95	2.22	8.91
Well done, USDA	72.8	23.5	158	1.90	0.61	4.13
Well done, Chef	25.5	4.14	74.2	0.66	0.11	1.94
Well done, Traditional	0	0	0	0	0	0
Pigs from non-controlled housing	Trichinellosis cases/year			Cases/million persons/year		
Scenario	Average	2.5 perc	97.5 perc	Average	2.5 perc	97.5 perc
Uncooked	318	0	878	8.30	0	22.9
Rare, Swart	308	0	853	8.05	0	22.2
Medim Swart	255	0	736	6.65	0	19.2
Well done Swart	153	0	478	3.99	0	12.5
Medium, USDA	140	0	445	3.65	0	11.6
Well done, USDA	77.3	0	271	2.02	0	7.06
Well done, Chef	38.7	0	163	1.01	0	4.25
Well done, Traditional	0	0	0	0	0	0

2.1.6.2. Freezing. The efficacy of freezing to kill *Trichinella* ML depends on freezing temperature, frozen storage time, *Trichinella* species and host species, but one week freezing at −18 °C or −21 °C effectively kills *T. spiralis* and *T. britovi* in infected meat (Hill et al., 2009; Lacour et al., 2013; Malakauskas and Kapel, 2003). This may affect human trichinellosis incidence, depending on usage of freezing to prolong

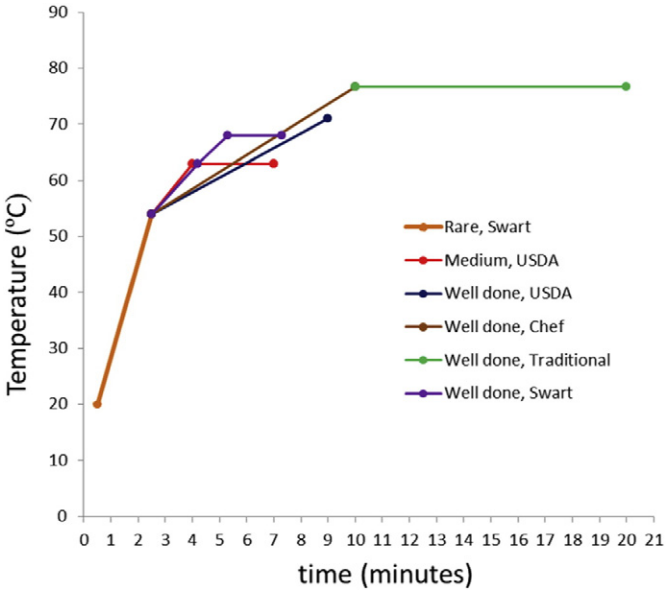

Fig. 3. Cooking scenario profiles. Overview of cooking scenarios built on basic scenario 'Rare', which runs from 20 °C until 53 °C. All other scenarios continue from there, except scenario 'Well done, Traditional', which continues from the final temperature of scenario 'Well done, Chef'.

shelf life of meat. However, since no data are available for Poland, inactivation by freezing was not included in the QMRA model.

2.1.7. Consumption patterns

Since consumption data for wild boar meat in Poland are lacking, we calculated the average number of consumable portions per person per year in that country as the total number of consumable portions of wild boar meat for the period 2007–2012, divided by the total population for Poland over the same period (Eq. (12)). The total number of consumable portions was calculated by multiplying the total number of tested wild boar over the period 2007–2012 by the total number of portions shoulder, loin and belly from a single wild boar (Table 5).

To calculate the total number of consumed portions of pork, first, the average number of consumed portions of pork weighing 100 g was calculated from consumption data during the period 2008–2013 (AHDB, 2015), resulting in 514 portions. Second, 233 out of 395 (59%) of portions from each carcass originate from shoulder, belly and loin, resulting in 314 portions. Finally, the adapted annual pork consumption in Poland was calculated using Eq. (13), taking into account that 78.4% of Polish pig farms keep their animals under non-controlled housing (Pozio, 2014).

2.1.8. Dose response modelling

Finally, to model disease risk from exposure to *Trichinella* ML, we used a previously published dose response model that includes a few different *Trichinella* species (Teunis et al., 2012) (Eq. (14)). This model takes into account the number of ingested larvae, but also the distribution of male and female worms that produce the next generation (new borne larvae), which cause disease. The output of the dose response model is the probability of illness, given exposure to a single portion of undercooked meat and presence of a known number of *Trichinella* ML in that meat.

2.2. Risk characterisation

The output of this module is distributions of human illness cases, simulated over a year, with each year a new realisation of the animal infection distribution (Eq. (1)). For each year, we simulate all portions from 5000 randomly generated carcasses. We modelled the average number of portions of undercooked pork or wild boar meat from false negative batches that cause illness, per million portions per year. Using this number and the consumption data for wild boar meat and pork as described above, the incidence rate due to consumption of pork or wild boar meat was calculated using Eq. (15) (wild boar) and Eq. (16) (pork from non-controlled housing), resulting in an estimate of the number of human trichinellosis cases per year. Finally, the incidence was divided by the population number (in millions) to generate incidence rate estimates, expressed as cases of human trichinellosis per million persons per year (Mpy).

2.3. Extrapolation to pigs from controlled housing

Seventy-eight percent of pigs produced in Poland are from non-controlled housing and data from controlled housing are unavailable. Hence, we resorted to data from the Netherlands, where the vast majority of pigs is kept under controlled housing. In the Netherlands, 139,729,393 pigs were tested in the period 2003–2013 and not a single pig tested positive during that period. It is not feasible to use these data for calculations in the present model because it is impossible to determine a measurable prevalence of *Trichinella* ML in these pigs, for use in the model. However, even if in the past this many pigs all tested negative, a future test may turn out positive. Therefore, we calculated the probability of finding one or more *Trichinella* positive pigs in 1 million (10% of the hypothetical slaughter volume) to 10 million pigs tested in the following year. All levels of prevalence between 0% and 100%, not a single level, contribute to the estimate on the probability, in proportion to the weight of evidence that is consistent with the observed zero positive animals out of 140 million tested animals.

Taking into account all the observed negative outcomes in the past, the beta distribution is the posterior probability distribution of a positive outcome for an additional test in the following year. For additional n tests in the following year, the number of positive outcomes is the beta binomial distribution (Eq. (17)). Assuming a uniform beta prior and taking into account 139,729,393 pigs tested in the past, we set two parameters of the beta binomial distribution at $A = 0$ (zero positives) and $B =$ 139,729,393 to model possible positive outcomes from testing an additional number of n pigs raised under controlled housing, with posterior *Beta* ($A + 1$, B-$A + 1$).

3. Results

Data from Polish *Trichinella* control at slaughter in the period 2007–2012 revealed an average *Trichinella* prevalence for wild boar of 0.0041 ($n = 685{,}595$, y = 2832, 95%CI 0.004–0.0043, Table 3) while for domestic pigs from non-controlled housing, the prevalence was 1.27×10^{-6} ($n = 114 \times 10^{6}$, y = 145, 95%CI 1.06×10^{-6}–1.48×10^{-6}, Table 3). The mean number of *Trichinella* ML in 50 g of diaphragm was 5.24 and 0.0016 for wild boar and pigs from non-controlled housing, respectively. *Trichinella* ML distribution among wild boars was 4.47×10^{-4} and 1.3523×10^{-7} in pigs from non-controlled housing (Table 3).

3.1. Trichinella testing at the meat inspection

The probability of recovering at least one *Trichinella* ML was estimated at 0.841 for one larva present in the test sample, 0.927 for two larvae, 0.956 for three larvae, rapidly increasing to 0.970–0.991 for 4–10 larvae that were present in the pooled test sample.

The estimated prevalence of false negatives in all pooled samples was 3.73×10^{-3} (95%CI 2.17×10^{-3}–4.9×10^{-3}) for wild boar and 6.03×10^{-6} (95% CI 0.00–1.65×10^{-5}) for domestic pigs from non-controlled housing (Table 8A). Only a minority of individual animals in these false negative pools was truly positive: 5.32×10^{-2} for wild boar (95%CI 5.00×10^{-2}–5.93×10^{-2}) and 0.010 for domestic pigs from non-controlled housing (95%CI 0.010–0.010) (Table 8B). Table 8C shows the prevalence of *Trichinella* ML infected portions for each of the modelled muscle types before cooking, over all portions of positive carcasses. The consumers cooked these infected portions.

3.2. Inactivation by cooking

Cooking meat to scenario 'Rare' hardly inactivates any *Trichinella* ML. We chose a combination scenario of 10% of portions cooked to 'Medium, USDA' and 90% of portions cooked to 'Well done Chef' to include consumer preference in the inactivation-by-cooking model. Results for this combination of cooking preferences for each of the muscle types are shown in Table 8D. The probability of illness for each of the portions from positive carcasses (from a false-negative batch) after cooking is given in Table 8E. Cooking scenario 'Well done, Traditional' is the only scenario that totally inactivates *Trichinella* ML in meat. However, we expect that this scenario applies to a minority of consumers.

3.3. Risk characterisation

An individual person on average consumed 0.68 portions of wild boar and 246 portions of pork shoulder, loin and belly each year.

3.3.1. Wild boar

The estimated annual prevalence of portions of wild boar potentially causing human illness was 2.89 (95%CI 1.21–5.88) per million cooked and consumed portions of shoulder, belly, or loin combined (Table 8G). This resulted in 75.3 (95%CI 31.6–153) cases of human

trichinellosis per year in Poland, or 1.97 (95%CI 0.82–4.00) human cases per million persons per year (Table 8K and L, respectively).

Not all Polish citizens will eat wild boar meat. Reported data from the Polish Hunters Association allow estimation of the sub-population of Polish hunters (approximately 100,000) and their families, about 400,000 persons in total (Rachubik, 2008), who will consume on average 65.4 portions of hunted wild boar meat. For this sub-population, we modelled on average 77.4 (95%CI 28.9–158) cases of human trichinellosis per year (data not shown).

3.3.2. Domestic pigs from non-controlled housing

The estimated annual prevalence of portions potentially causing human trichinellosis per million portions of pork from pigs from non-controlled housing was 0.004 (95%CI: 0–0.013) per million cooked and consumed portions of shoulder, belly, and loin combined (Table 8G). This was equivalent to 34.6 (95%CI 0–141) cases of human trichinellosis for the Polish situation, or 0.904 (95%CI 0–3.68) cases of trichinellosis per million persons per year Table 8 (K and L, respectively).

3.3.3. Risk analysis for different cooking scenarios

All cooking scenarios were evaluated against scenario 'Rare, Swart' using estimated annual human trichinellosis as a measure (Table 7, Fig. 3). In each scenario, the same cooking methodology applies to all edible portions. Resulting incidence estimates for each of the modelled cooking scenarios for both wild boar and pigs from non-controlled housing are shown in Table 9. These results allow quantification of the effect of cooking to prevent human trichinellosis. Without cooking, the hypothetical number of cases of trichinellosis per year from consumption of wild boar meat, is estimated 747 (95%CI 430–1120), and 318 (95%CI 0–878) for consumption of pork from non-controlled housing.

3.3.4. Comparison with epidemiologic estimates

Incidence rate estimates from our model are 1.97 per Mpy for wild boar and 0.904 per Mpy for pigs from non-controlled housing (Table 8L), which results in a total incidence rate estimate of 2.87 cases of human trichinellosis per million inhabitants per year. The observed incidence rate for Poland for the period 2007–2012 is 1.15 Mpy (EFSA-ECDC, 2010, 2013, 2014). Our estimate is therefore not inconsistent with best-available independent evidence, although direct comparison between the epidemiological evidence and our model is not straightforward (see the Discussion).

3.3.5. Extrapolation to pigs from controlled housing

In view of the 139,729,393 test-negative pigs from controlled housing in the Netherlands, the probability of finding *Trichinella* ML in future tests is minute. At a theoretical estimate, given these negative pigs so far, at a scenario slaughter volume of 10 million pigs in the next year, the probability of finding one positive pig out of 1 million tested is 0.0007. It takes testing of 7.3 million pigs to achieve a probability of 0.05 of finding one or more positive pigs, but even considering all 10 million hypothetical pigs, the probability of finding one or more positive pig is still only 0.067.

Alternatively, EU data enabled us to better estimate the upper prevalence limit, since far more pigs from controlled housing have been tested in the past. Roughly 120 million pigs from controlled housing are slaughtered and tested annually in the European Union (Pozio, 2014), without any *Trichinella* ML findings in the last two decades. As a result, the observed EU-wide prevalence is less than one per 2400 million pigs from controlled housing. From this data, an upper limit of prevalence for pigs from controlled housing is estimated (4.2×10^{-10}) using Eq. (18). From the upper prevalence limit, we estimated that the risk from eating pork from controlled housing is $<5.3 \times 10^{-4}$ human cases per million per year (Eq. (19)), or less than one citizen of the European Union in 4 years, assuming a linear relationship between prevalence and incidence.

4. Discussion

In this paper we present a quantitative microbial risk assessment (QMRA) model for *Trichinella*, based on experimental and literature data. We modelled human trichinellosis incidence from consumption of both wild boar and domestic pig meat from non-controlled housing, using Poland as an example. We implemented in our model the distribution of *Trichinella* spp. numbers in meat, inactivation of *Trichinella* ML by cooking and a dose-response relationships for *Trichinella* spp. infections in humans.

Infected swine that escape carcass control pose a risk for human trichinellosis. In the present study, we assumed the lower limit for *Trichinella* ML detection at meat inspection to be one larva per gram, representing one positive animal in a pooled sample, the lowest theoretically achievable detection level using the magnetic stirrer test, which is the standard reference method (European-Commission, 2015). Our estimates on the probability of finding one larva in a 100 g pooled sample are consistent with a previous study using experimentally infected pigs, which reported a test sensitivity of 73% for pooled digestion of 100 samples of 1 g at an infection level of 1.0–1.4 larvae per gram (Forbes and Gajadhar, 1999). Sensitivity in that study when testing a lower infection level (0.001–0.9 larvae per gram) dropped to 40%, which is understandable in view of high probability of missing larvae at this level. It is precisely this situation where we expect our model to perform well. Indeed, our model predicts a range between 1 and 500 *Trichinella* muscle larvae per 100 g of diaphragm (on average 0.01–5 larvae per gram) of positive animals that were missed at meat inspection and allows subsequent estimation of resulting cases of human trichinellosis.

Our estimates are based on data regarding the hazard in Poland, and therefore the reported human trichinellosis incidence rate in Poland (1.15 human cases/Mpy) should be the most compatible, alternative measure of risk. Comparable magnitude in our QMRA estimates and in the reported incidence rate is a fair support for our modelling approach. Our QMRA model shows an estimated annual incidence rate in Poland of 75.3 (95%CI 31.6–153 cases, 1.97 cases/Mpy) from consumption of wild boar. For consumption of pork from pigs reared under non-controlled housing, the estimated incidence rate was 34.6 (95%CI 0.00–141 cases, 0.904 cases/Mpy). In our QMRA model we corrected the number of consumed portions for muscle type (shoulder, loin and belly) and for the proportion of pigs from non-controlled housing. However, an unknown part of pork will be consumed in the form of heat processed products, which may reduce the modelled incidence from pigs under non-controlled housing by a factor 2–3, to 11.5–17.3 cases (0.301–0.452 cases/Mpy).

Our QMRA model includes all stages in the chain of events from *Trichinella* prevalence and distribution in animals to exposure, infection and illness in humans. This allows quantification of risk when one or more parts in the chain change due to a locally-specific factor, such as country-specific *Trichinella* prevalence and abundance, varying test sensitivity at meat inspection, different proportions of controlled housing in a country, and consumption data.

However, we did not include some factors that might influence the results, such as consumption of raw meat products. The term 'raw meat products' refers to an inhomogeneous group of different product types, varying from dried or smoked ham to complicated, combined products, such as sausages, each with its own preparation method (Savic, 1985). Industrially produced raw sausages are prepared from *Trichinella* tested and certified pork, following strict regulations, surrounded by QA measures, to guarantee efficient inactivation of *Trichinella* muscle larva and other potential pathogens that might be present in the raw meat (Essien, 2003; Porto-Fett et al., 2010; Smith et al., 1989). In contrast, fresh home-made raw sausages, one of the main

causes for trichinellosis in Europe, are often prepared from non-tested wild boar meat or non-tested pork from back-yard pigs (Sadkowska-Todys and Golab, 2013). Typically, these sausages are shared with family and friends, and often cause small and clustered outbreaks, not only in Poland, but also in other *Trichinella*-endemic countries (Neghina, 2010). Eight outbreaks due to consumption of *Trichinella* infested raw wild boar sausages have been described in seven European countries (including Poland) between 2007 and 2015, causing 7–219 confirmed human cases per outbreak (Bartuliene et al., 2009; Fichi et al., 2015; Gallardo et al., 2007; Golab et al., 2007; Nockler et al., 2007).

To evaluate the contribution magnitude of raw sausages to incidence of human trichinellosis, we modelled a crude approximation. For this purpose, we hypothesized a scenario in which 10% of edible portions of a carcass would be used for sausage production, a portion size of fresh raw sausage of 200 g (containing 50% meat besides other ingredients, e.g. water, fat, spices), 30% inactivation of *Trichinella* ML during fermentation and a (sub)population at risk of 400,000 (hunters and their families). This resulted in 52.7 (95%CI 24.2–88.5) cases, or 1.32 (95%CI 0.61–2.21) cases per 10,000 persons per year for this subpopulation.

The reported total number of human trichinellosis cases in Poland over the period 2007–2012(EFSA-ECDC, 2010, 2013, 2014), ranged between 1 and 216 confirmed cases per year for the whole population. The reported numbers appear congruent to the sum of our estimates. Note however, that our model predicts the number of sporadic cases, while the reported number includes clustered outbreaks of trichinellosis. However, as the number of infected animals will be low, also the disease cases modelled here will be clustered, even though this is not explicit in our model.

For the sake of clarity, we did not include heterogeneity into all aspects of the QMRA model. One example of heterogeneity that is not included in the presented model is consumption pattern in a country, where a group of non-pork consumers may be present. A recently published consumer survey, conducted in 2008 in five European countries (Belgium, Denmark, Germany, Greece and Poland), showed that 12% of respondents (mostly women living alone) never eat pork. The survey reported in addition: 18% rarely eat pork (predominantly single women), 51% eat one serving of fresh or processed pork per day (families and other non-single households), and 19% eat several servings per day of both fresh and processed pork (predominantly less educated overweight males) (Verbeke et al., 2010). In our approach, all persons in the population are assumed to consume slightly more than one portion of pork per day. The current model would need further development to evaluate the effect of a combination of different consumption patterns.

Another example of heterogeneity that is not included in the presented model is consumable portions weighing >100 g. The per capita pork consumption in Western Europe ranged from 24.1 kg (UK) to 57.6 kg (Austria) in the years 2008–2013, indicating a pork consumption of on average 66–158 g per capita per day. The highest average pork consumption was recorded in Austria, Germany, Poland and Spain (50.2–57.6 kg) (AHDB, 2015). The average pork consumption does not discriminate between groups within a society and the actual portion size may be higher: the young and the elderly generally eat less meat, and men generally eat more than women do. Perhaps the actual portion size per occasion might well be 100–300 g. Papers describing trichinellosis outbreaks, report portion sizes of 58–396 g reviewed in (Teunis et al., 2012).

We did include heterogeneity in consumer preferences to prepare a meal in an accustomed way. We chose a combination of 10% of portions cooked to scenario 'Medium, USDA' and 90% of portions cooked to 'Well done Chef' to model inactivation by cooking. In a published study using experimental pan frying of different types of meat, pork chops cooked for 6.5 min to an internal temperature of 70 °C, were considered well done (Lahou et al., 2015). This approaches our cooking scenario 'Well done, USDA' and may be an alternative to 'Well done Chef' in our setting. However, Lahou and co-workers state that in three replicate experiments, their scenario failed to achieve an internal temperature of 70 °C for two minutes for pork chops, which they considered necessary to render the meat microbiologically safe. This implies that 6.5 min cooking time may be too short, which supports the longer cooking time in both scenario 'Well done, USDA' and 'Well done Chef' in our setting. Inclusion of a third scenario into our QMRA may be needed to achieve a more accurate approximation of consumer preference, since we used scenario 'Medium, Swart' to include consumption of meat that has pink or red juices into the model, the latter of which may implicate lower doneness than achieved with scenario 'Medium, Swart'.

We did not include *Trichinella* inactivation through home freezing by consumers, who will probably freeze a proportion of purchased fresh pork or wild boar meat, to prolong its shelf life. The extent to which this takes place is difficult to establish. In a British survey, 80% of households own a consumer freezer, of which 51% would freeze fresh meat (not specifically pork) (Maxey and Oliver, 2010). In a Scottish high-income consumer survey, 60% of responders would freeze wild game meat (FSAS, 2012). If a proportion of consumers would freeze fresh meat, this could proportionally lower human exposure to *Trichinella* ML and the resulting incidence of human trichinellosis estimated in our model. More research is needed to establish to which extent consumers actually do freeze pork or wild boar meat, and whether these home-freezing conditions fully inactivate *Trichinella* ML.

More work is needed to evaluate uncertainty in some other parts of our model. e.g. for weight of diaphragm samples, we used the required weight according to EU Regulation 2015/1375, whereas the actual weight may vary and depends on correct functioning of sampling devices. *Trichinella* ML prevalence in domestic pigs from non-controlled housing and in wild boar is another example. Since we did not have data concerning test sensitivity in Polish slaughterhouse labs during the seven year period used in our model, we used prevalence data as reported by EFSA, regardless of test sensitivity. As a result, the prevalence in domestic pigs and wild boars may have been underestimated in our model. Cooking, which is a highly subjective factor that may harbour considerable uncertainty, is yet another example, since most people will use a rough combination of time, estimated cooking heat and meat weight, to reach a certain doneness of the cooked meat. As a result, the actual temperature inactivation of *Trichinella* ML that may be achieved is highly variable, as illustrated in Table 9.

In the present paper, we estimated that the risk from eating pork from controlled housing is $<5.3 \times 10^{-4}$ human cases per million per year or less than one citizen of the European Union with trichinellosis due to eating pork from controlled housing in 4 years. This corroborates derogation from *Trichinella* testing for pigs that are reared under controlled housing and justifies alternative surveillance strategies for animals from this type of husbandry. EU Regulation 2015/1375 (European-Commission, 2015) (Article 2a) stipulates that under controlled housing, at least 10% of the annually slaughtered pigs should be tested, among which all sows and boars, which have a longer life and therefore a higher theoretical risk of infection with *Trichinella*. However, in view of complete absence of positive findings at meat inspection, testing of 10% of animals from controlled housing to demonstrate the absence of *Trichinella* is questionable. In the Dutch example, described in this paper, at a volume of 140 million *Trichinella*-negative pigs at meat inspection in ten preceding years, the probability of finding a positive pig is not >0.0007 if testing 10% of pigs from controlled housing, including sows and boars. In the Dutch example, testing of about 7.3 million pigs (50–60% of the annual slaughter volume) is needed to obtain a 5% probability of finding a positive pig. Under approved controlled housing as shown here for the Dutch situation, *Trichinella* testing is not adding any value to protect human health, although for the time being, 100% of Dutch slaughter pigs will remain tested for *Trichinella*, due to export requirements.

In conclusion, our QMRA model provides estimates for human trichinellosis incidence from different meat production systems.

Comparable magnitude in our QMRA estimates and reported incidence rate, supports the validity of our modelling approach. Our model may prove useful to evaluate alternative scenarios for the quantification of regional consumer-related variables, such as meat consumption, meat portion size and cooking habits, raw sausage consumption and *Trichinella* inactivation through home-freezing of pork, as well as production system-related variables. Finally, our QMRA model may be used to support development of meaningful risk-based monitoring programmes to control *Trichinella* in pigs from different housing systems.

Conflict of interest

The authors declare no conflict of interests.

Acknowledgements

The authors thank Ewa Bilska for sharing Polish wild boar test data; Frans van Knapen and Peter Teunis are thanked for critically reading the manuscript. Anne Mayer-Scholl is thanked for sharing experimental data and for critically reading the manuscript. Parts of this study were financed by the Netherlands Food and Product Safety Authority.

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