Human beings are highly susceptible to low doses of *Trichinella* spp.

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Abstract

Trichinella is an important foodborne pathogen causing considerable morbidity and mortality. To prevent human trichinellosis, meat inspection for *Trichinella* spp. at slaughter is a key instrument. Current testing is based on minimal infectious dose in humans, but a scientific basis for this approach is lacking. To this end, a dose response model must be developed, allowing translation of exposure into disease burden at the population level.

We have developed novel methods for dose response assessment using outbreak data incorporating sexual reproduction of the parasite. A selection of suitable outbreak studies, reporting numbers exposed and infected, as well as estimated doses, has been collated from a literature study.

Humans appear to be highly susceptible: exposure to low doses (few larvae) is associated with a considerable risk of infection. As a consequence, levels of *Trichinella* in meat must be low to maintain acceptable health risks.

Introduction

Trichinellosis is a zoonotic disease that is caused by nematode parasites of the genus *Trichinella*. The life cycle can take place in many different carnivorous —and omnivorous animal species, including humans, by infection after oral ingestion of infective larvae present in striated muscles of infected animals. Infective larvae develop in the epithelial cells of the small intestines to adult male and female parasites and after mating, newborn larvae migrate via blood and lymphatic vessels to the predilection sites and finally encapsulate in striated muscle cells ready to infect the next host.

In Europe, the most important sources for human infection are improp-

erly processed infected meat of domestic pig, horses and wild boars. Since trichinellosis is considered a serious disease in humans, meat inspection (based on the testing of an appropriate amount of meat sampled after slaughtering) is a historical keystone in European policy for preventing clinical symptoms in humans. This is particularly relevant for international trade of meat and meat products because of regional differences in the prevalence of this parasite in Europe. All meats marketed for human consumption must be inspected for larval *Trichinella* in the EU [1]. Testing is based on risk assessment to prevent human clinical illness by the assumption that the minimal infectious dose for humans is between 60 and 750 infective larvae [1] and thus testing should indicate the absence of *Trichinella* in one gram of pig meat and 5 grams of horse or wild boar meat, considering an average human portion of 100 grams of meat. Clinical illness in humans is dependent on the dose ingested, and can range from asymptomatic to severe illness and mortality, however, scientific evidence of the dose response in humans is still lacking.

The first step in estimating the health risks associated with *Trichinella* in meat and meat products is assessment of human exposure to living parasites, viable for infection. When the dose is known, health effects resulting from exposure must be quantified. A dose response relation quantitatively describes the probability of infection and/or illness as a function of exposure. Quantitative risk assessment should incorporate an empirical assessment of human susceptibility. When the dose response relation is known any exposure (estimated number of larvae ingested) can be translated into a probability that this exposure causes infection. Conversely, any arbitrary level of risk can be translated into a corresponding level of exposure, aiding

the setting of standards e.g. by regulating agencies.

For several microbial pathogens dose response assessment has been based on human challenge studies, in which human volunteers have ingested defined doses of pathogens. This is only ethically acceptable for mild pathogens that do not cause much discomfort, and certainly do not inflict much damage on the volunteers. The seriousness of the symptoms caused by *Trichinella* makes it unlikely that volunteer studies will be done in humans.

There are however many reported outbreaks of trichinellosis in humans. In some of these outbreaks a sample of the contaminated food was available for analysis, and some of these did produce a useful count of the number of larvae. Such occurrences may be used to assess the dose response relation [2, 3].

A literature study, combined with a questionnaire to participants of the Trichimed network, produced a set of usable outbreaks, shown below. We show that these outbreak reports allow estimation of the infectivity of *Trichinella* in humans, in turn making human risk assessment possible.

Materials and Methods

Data from *Trichinella* outbreaks

In order to be used for dose response assessment, outbreak reports must at least provide data on numbers of subjects exposed to the contaminated food, and numbers of subjects who developed symptoms. Further, information allowing estimation of the ingested dose must be present: concentration of larvae in the contaminated food and the amount of food that was consumed by exposed persons.

Preferably, these data of exposure should allow assessment of heterogeneity in exposure. Some subjects may have eaten more than others, and some parts of the infected animal may have contained more larvae than other parts. Information of such factors determining heterogeneity in exposure increases the usefulness of the data.

Fortunately, suspected cases of trichinellosis are often tested serologically, and in a cluster of cases asymptomatic subjects who also consumed infected meat are tested as well. In principle, this allows detection of asymptomatic infections: serologically positive (seroconverting) individuals without symptoms. In most of the incidents used here, all infected subjects became ill, and we conclude that human exposure nearly always results in illness.

Considerable numbers of outbreaks or incidents of trichinellosis are reported, but only a small proportion of these produced the necessary information for quantitative study of infectivity. We succeeded in collecting 9 outbreaks over a period of 6 years (2000–2006).

Dose response model

Muscles can become infected with larvae if and only if 1 or more female pathogens and 1 or more male pathogens survive. If female and male pathogens have equal survival probabilities p_m, and female and male pathogens are present in proportions r and $1 - r$ (r is the sex ratio: the fraction female larvae), then the probability of infection is

$$P_{\text{inf}}(C \cdot V | p_m, r) = 1 + \mathrm{e}^{-C \cdot V p_m} - \mathrm{e}^{-C \cdot V p_m (1-r)} - \mathrm{e}^{-C \cdot V p_m r} \qquad (1)$$

assuming Poisson exposure with dose CV: intake of volume V with concentration C pathogens per unit volume (or mass).

In case of a sexual reproduction cycle, the dose response relation can thus be expressed as a linear combination of three terms, each equivalent to the simple exponential dose response model for asexual pathogens [4].

For heterogeneous (Beta distributed) p_m can again be written as a linear combination, of confluent hypergeometric functions [2]

$$P_{\text{inf}}(C \cdot V|\alpha, \beta, r) = 1 + {}_1F_1\left[\alpha, \alpha+\beta; -C \cdot V\right] - {}_1F_1\left[\alpha, \alpha+\beta; -C \cdot V(1-r)\right] - {}_1F_1\left[\alpha, \alpha+\beta; -C \cdot Vr\right] \quad (2)$$

In case the dose also has extra–Poisson variation, with dispersion factor ρ [3], the resulting dose response relation is a linear combination of another hypergeometric function

$$P_{\text{inf}}(\rho, \tilde{c}, V|\alpha, \beta, r) = 1 + {}_2F_1(\alpha, \rho, \alpha+\beta; -\tilde{c}V/\rho) - {}_2F_1(\alpha, \rho, \alpha+\beta; -\tilde{c}V(1-r)/\rho) - {}_2F_1(\alpha, \rho, \alpha+\beta; -\tilde{c}Vr/\rho) \quad (3)$$

The influence of the sex ratio r on the shape of the dose response relation is strongest when its value is close to 0.5 . Such values of r tend to steepen the slope of the dose response relation. For extreme values (either near 0 or 1) there is a shortage of females or males, which then becomes the limiting factor in infection and acts in downscaling the dose (shifting the dose response relation to the right on the dose axis, without changing its shape).

Statistical methods

The likelihood is binomial: for each incident K out of N subjects exposed to a dose $D = g(\rho, \tilde{c}, V)$ have been observed to be affected.

Given the hit theory dose response relation, a single observed fraction infected (i.e. response) may allow prediction of the dose response relation [2]. We want to incorporate data from several outbreaks with different levels of exposure, and a single response rate at each dose. However, such an approach inevitably involves an additional level of biological variation. While a different human population similar in age and health status might have similar susceptibility, a different isolate of the pathogen is likely to have completely different infectivity, if only because of a different history (different food vehicle, different previous host). Therefore, analysis of data from different outbreaks requires a hierarchical model.

The dose is characterized by the expected concentration of pathogens, and their dispersion, characterized by the Gamma shape parameter ρ[3]. These two parameters (dose and dispersion parameter) were estimated separately using whatever information was available in the outbreak reports. In most outbreaks reports a range was provided for the intake of contaminated unheated (or inadequately heated) meat.

Results

Outbreak data

For all used outbreaks, a number of *Trichinella* larvae per g contaminated meat was reported. Also, some information on the intake of contaminated

meat was available, varying from a direct indication of the consumed amount to information on the type of meal in which the meat was used.

Table 1 here.

Table 1 lists this information for the admissible outbreak reports. Additional details follow below:

Ranque et al. 2000 [5]: Four human cases exposed to *Trichinella pseudospiralis* in France, two patients ate less than 300 g, the other two ate more than 300 g wild boar meat with estimated 187 larvae/g. **Pozio et al. 2006 [6]**: Eleven people ate raw sausages containing 8 larvae/g of *Trichinella britovi*, 10 were symptomatic, 1 asymptomatic. **Gari–Toussaint et al. 2005 [7]**: Six people consumed frozen wild boar meat with 3 larvae/g of *Trichinella britovi*, all were infected (all symptomatic). **Turk et al. 2006 [8]**: At a wedding 474 people ate raw meatballs with 6.5 larvae/g of *Trichinella britovi*. Of these, 154 were confirmed with trichinellosis, of the remaining exposed, 71 were initially diagnosed with highly likely trichinellosis, 60 with probable trichinellosis, 42 with suspected trichinellosis, and 147 with highly unlikely trichinellosis. All these latter subjects appeared to be seronegative. **Ancelle et al. 2006 [9]**: A bear shot in Canada appeared to be highly infected with 295 larvae/g of *Trichinella nativa*, resulting in three clusters of cases. Of the 10 hunters who all consumed meat, 8 developed clinical disease. In Orleans 6 people ate the same meat, 5 of which became ill. And two weeks later in Narbonne, 9 people ate of the same meat, and 4 of these became infected and ill. At the time of the last cluster, the first patients had been identified and treated. The cases in Narbonne were also treated at the same time, shortly after they had been exposed. Therefore this latter cluster may show lower infectivity, as some of the exposed subjects may have been saved

from developing infection. **Littman et al. 2006 [10]**: Consumption of meat and meat products from a home–reared pig containing approximately 106 larvae/g of *Trichinella spiralis* by 22 people resulted in 18 infected cases, 17 of whom symptomatic. **Rouen 2004 (Table A1)**: Black bear meat infected with *Trichinella nativa*; 1 case, consumed 300 g containing 250 larvae/g. **Nans les pins 2006 (Table A1)**: Three cases caused by consumption of wild boar meat with approximately 40 larvae/g of *Trichinella spiralis*. **Collobriëres (Table A1)**: Ten people consumed wild boar meat with 5–10 larvae/g of *Trichinella britovi*, 4 symptomatic.

Table 2 shows exposure parameters and response data for the 9 outbreaks included.

Table 2 here.

Studies of the sex ratio of trichinellae in the intestines are rare; Christenson [11] fed rats with meat containing high numbers of larvae of *Trichinella spiralis* and initially (after challenge) found equal numbers of female and male worms. A few days later the fraction males started to decrease, as did total numbers of worms in intestinal contents. Gursch [12] also observed decreasing numbers of intestinal worms as infection (*T. spiralis* larvae migrating into muscle tissue) progressed, but found that initially females outnumbered males by a factor of about 2:1, while later (10–14 days) the sex ratio decreased. In a study in hamsters Boyd et al. [13] found similar results: initial ratios close to 2:1, later (6 days) decreasing to 1.5:1. In a more recent study Leiby et al. [14] found similar sex ratios in mice infected with *T.spiralis* larvae from three different sources. Therefore we will here use a fraction of 0.7 females (and 0.3 males), corresponding to a sex ratio of about 2:1.

Dose response relations

Figure 1 here.

Figure 1 shows fitted dose response relations (equation 3) for each of the included outbreaks. The variation in location of the curves illustrates possible biological variation in infectivity of larvae from different sources, in different host populations, and in different food media. Also clearly visible in this graph is the difference in slopes of the curves, indicating heterogeneity from a combination of pathogen factors (differences in within outbreak variation in infectivity) and inoculum factors (differences in extra–Poisson variation in pathogen occurrence). The Narbonne cluster [9], where the attack rate is substantially lower than in the two remaining clusters from the same outbreak, has been left out.

Figure 2 here.

The gray area in Figure 1 is a density graph of predicted infectivities, generalizing over all included outbreaks. This distribution is obtained by sampling from the "group" (prior) distributions for the infectivity parameters α and β and it represents the infectivity of an unspecified *Trichinella* inoculum with properties as described by the collection of outbreaks used. This distribution may be used for prediction of the risk of infection.

For the predicted dose response relations the inoculum is assumed Poisson (i.e. no extra–Poisson variation, or dispersion $\rho \gg 10$). Of course, for any particular exposure scenario assuming dispersion, simulations may include any degree of dispersion required.

Figure 2 shows the a density graph of the distribution of p_m, the survival probability of individual larvae. The shading corresponds to that in Figure

1.

Table 3 here.

Metrics derived from the fitted outbreak dose response model are listed in Table 3. These illustrate in particular the low dose behaviour of the model: as the single hit probability p_m is approximately 0.01, exposure to exactly 1 male and 1 female larva is expected to lead to infection with a probability of $0.01 \times 0.01 = 10^{-4}$. Although the infection risk at high doses (> 10 larvae) is considerable, with decreasing doses the risk rapidly decreases, so that at a dose of 0.1 (exposure to 1 larva, in each 10 exposure events, approximately) the risk is quite low (97.5% level near 1 in 10,000).

Discussion

Prime evidence for the human health effects of any infectious micro–organism is found in outbreaks. If outbreak studies succeed in obtaining data on exposure of cases, they may be interpreted as 'natural experiments', and may be used for dose response assessment. Compared to previous studies of bacterial infectivity based on human outbreak data [2, 15, 3], *Trichinella* is interesting because infection requires the presence of both a female and a male parasite. The necessary adaptations to the mathematical model used for dose response assessment cause the relation to change shape. Compared to a 'non-sexual' dose response model the relation is steeper, dependent on the sex ratio of the parasites. The parametrization of the dose response model represents useful *a priori* information when data are not very informative of the shape of the relation [4].

Based on the collection of outbreaks we conclude that the infectivity of *Trichinella* in humans is high, ranging from a likely value of 0.01 to an upper (95%) level near 0.1 (Figure 2): only a few pairs of *Trichinella* larvae are needed to achieve a considerable probability of infection. The median 50% infectious dose is close to 150 larvae (Table 3a).

As a consequence, safe levels of *Trichinella* larvae in meat corresponding to acceptable risk levels should be low, limiting the average individual yearly dose to less than 0.1, for instance (thereby limiting individual yearly risk to less than 1 in 10,000 approximately). For any single exposure event, even ingestion of a few *Trichinella* larvae already represents a considerable risk of infection of a few per cent (Table 3b).

Table 4 here.

Routine testing surveillance in the Netherlands consists of taking pooled samples of size 1 g from one hundred slaughtered pigs and inspecting these for the presence of *Trichinella* larvae after digestion of the meat tissue. Assuming the digestion test has a probability of failing (varying as a beta distribution with parameters (α, β) = (1.0,1.5) so that the average sensitivity is 40%) and heterogeneous (gamma distributed) concentration of larvae in meat, the infection risk associated with consumption of 100 g undercooked meat may be calculated, as a function of the numbers of meat samples analyzed. With increasing numbers of samples, the risk rapidly decreases to low (acceptable) levels, as shown in Table 4. In the past years, millions of meat samples have been tested, so that infection risks are low.

In contrast to waterborne exposure, pathogens in food cannot usually be assumed well dispersed. In agreement with methods reported in an earlier paper [3], we estimated average doses from the average concentration of

larvae in the foodstuff and the average quantity consumed. All heterogeneity in exposure, due to uneven distribution of larvae in the foodstuff as well as variation in the consumed portions among human cases, was summarized in a dispersion coefficient (ρ). Such dispersion also is *a priori* information, that can be inserted into the dose response relation, helping to determine its shape.

Compared to a simple, straightforward dose response assessment we have accounted for three sources of variation: (1) requirement of sexual reproduction for infection; (2) overdispersion in exposure due to inhomogeneous distribution of larvae and variable consumption; and (3) variation in infectivity among outbreaks, due to different *Trichinella* species, differences in susceptibility of cases and other (unspecified) variation in exposure. The latter factor is accounted for by the hierarchical structure of the model.

Although in all outbreaks the infected meat must have contained viable larvae, some die–off may have occurred. In one of the outbreaks [7] the meat had been frozen at -35C for 7 days, and most of the other incidents involved heating. Doses may therefore have been overestimated, if the larvae were counted in a sample of the raw meat instead of the consumed meal. That would mean that the infectivity estimates would be biased downward. As our analysis indicates very high infectivity, the error caused by underestimation of the dose is not likely to be considerable.

Detection of asymptomatic cases in outbreaks is usually a difficult problem. In most of the selected outbreaks used here, blood samples were collected, not only from cases, but also from all exposed subjects. This allows the use of seroconversion as an indicator of infection, including asymptomatic infections. It appears that the fraction of infected cases who were

symptomatic is quite high: 99% (212 out of 214 total), indicating that the conditional probability of becoming ill when infected with *Trichinella* may be very high. Our dose response model therefore does not distinguish between symptomatic and asymptomatic infections, assuming all infections are symptomatic.

Although animal models of infection and disease are important in clinical research, dose response studies with animals are not well suited for human risk assessment. The purpose of a dose response model is to not just establish a causal relation between dose and probability of infection, but also quantification of that probability. Even when an animal model shows an appropriate response (comparable symptoms, for instance), there is little reason to trust that the magnitude of such a response would be the same as in a human host.

An experimental study of *Trichinella* infection in pigs [16] showed lower infectivities, indicating that humans may be more susceptible to infection with this parasite than pigs. Recent experimental infections in mice to establish the dose response relation [17] also showed high susceptibility, different from pigs. In pigs, different infectivities have been found for different *Trichinella* species [18, 19, 20]. Human susceptibility to symptomatic infection was high in all observed outbreaks, in spite of the fact that these involved various species of *Trichinella*. Little variation may be seen if the susceptibility to each species is so high that infection is probable, given even minimal exposure [4]. We recognize the possibility, however, that the actual range of infectivity among and within these parasite species may exceed that represented in the present sample of highly infectious types.

Outbreaks caused by highly infectious, highly pathogenic parasites are

more likely to be detected than those caused by milder parasites. The selection of outbreaks used for dose response assessment may therefore be biased towards those more infectious parasites, possibly overestimating the risk in the general population. The collected outbreaks show a variety of infectious *Trichinella* spp., so that use of outbreak based infectivity estimates seems a prudent approach, using the precautionary principle of risk assessment [21].

The dose response model developed here may be used either in studies of human risk of trichinellosis and to assess testing criteria for slaughtered animals to prevent human cases. Exposure estimates can be translated into estimated probabilities of infection and illness, or projected numbers of cases for specific exposure scenarios can be calculated. For this purpose, the predicted dose response relations (the grey area shown in Figure 1) may be used, as they represent the infectivity of a hypothetical isolate, as a sample from a population like the selected collection of outbreaks. The present study is based on a relatively small number of outbreaks, not allowing stratification for pathogen type (species), food type (preparation, storage) or host properties (age, proxies for immune competence). To further specify the dose response relation of *Trichinella* in humans, more outbreak data are needed, preferably with reliable information on the numbers of larvae present in the contaminated food.

One might discuss whether the current method of testing is sufficiently sensitive to prevent human cases. Currently, susceptible animal species destined for human consumption must be tested for the absence of *Trichinella* spp. at slaughter or at game handling plants in the European Union (EU regulations). The method prescribed for routine testing of pig meat is artificial digestion, which is typically applied by pooling up to one hundred samples

of at least one gram of meat. However, this reference test has limitations in terms of diagnostic and analytical sensitivity and it was demonstrated that reliable detection of *Trichinella* positive samples was only guaranteed when samples contained more than 3 larvae per gram [22]. For this reason meat from pigs tested negative might be infected with higher numbers of larvae than considered safe for consumption. Our dose response model even shows that the consumption of 100 grams of pork infected with 200 larvae (LPG 2) might be tested negative by the approved method but can be considered unsafe for human consumption since the probability of disease after ingestion of 200 larvae is considerable. Based on the present study, it may therefore be recommended to reconsider the current testing protocol.

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Tables

Reference	species	Conc. (larvae/g)	Intake Percentiles (g)	
Ranque et al. [5]	*pseudospiralis*	187.0	$P_{0.05}$ = 75 g	$P_{0.90}$ = 300 g
	pseudospiralis	187.0	$P_{0.10}$ = 300 g	$P_{0.90}$ = 500 g
Pozio et al. [6]	*britovi*	8.0	$P_{0.05}$ = 75 g	$P_{0.90}$ = 300 g
Gari–Toussaint et al. [7]	*britovi*	3.0	$P_{0.10}$ = 100 g	$P_{0.90}$ = 300 g
Turk et al. [8]	*britovi*	6.5	$P_{0.01}$ = 25 g	$P_{0.90}$ = 100 g
Ancelle et al. [9]	*nativa*	295.0	$P_{0.20}$ = 100 g	$P_{0.80}$ = 200 g
Littman et al. [10]	*spiralis*	106.0	$P_{0.20}$ = 50 g	$P_{0.80}$ = 100 g
Table A1 Rouen (2004)	*nativa*	250.0	$P_{0.20}$ = 250 g	$P_{0.80}$ = 350 g
Table A1 Nans les Pins (2006)	*spiralis*	40.0	$P_{0.20}$ = 150 g	$P_{0.80}$ = 250 g
Table A1 Collobriëres (2006)	*britovi*	5.0–10.0	$P_{0.20}$ = 125 g	$P_{0.80}$ = 175 g

Table 1: Data used for exposure assessment. The concentration of larvae in the implicated meat has been determined from stored samples. To quantify the distribution of the amounts of contaminated meat consumed lower and upper limits have been determined as percentiles.

Reference	Dose conc (larvae/g)	Dose intake (g)	Dose disp (ρ)	Response Exposed	Response Infected	Response Symptoms
Ranque et al. [5]	187.0	188.3	5.09	2	2	2
	187.0	396.7	25.50	2	2	2
Pozio et al. [6]	8.0	188.3	5.09	11	11	10
Gari–Toussaint et al. [7]	3.0	192.9	5.80	6	6	6
Turk et al. [8]	6.5	58.0	8.13	474	154	154
Ancelle et al. [9]	299.8	152.3	4.60	16	13	13
	299.8	152.3	4.60	9[a]	4[a]	4[a]
Littman et al. [10]	111.8	76.2	2.90	22	18	17
Table A1 Rouen (2004)	251.4	301.2	17.49	1	1	1
Table A1 Nans les Pins (2006)	40.4	201.7	8.36	3	3	3
Table A1 Collobriëres (2006)	7.6	150.6	4.83	10	4	4

[a]Narbonne cluster, not included

Table 2: Outbreak data used for dose response assessment. Exposure characterized by larvae concentration in contaminated meat, average intake and extra–Poisson dispersion parameter (ρ). Infection characterized by elevated levels of antibodies against *Trichinella*.

(a) Dose required for 50% and 1% infection

	Mean	Median	$Q_{0.025}$	$Q_{0.975}$
ID_{50}	260.0	135.5	11.0	3.79×10^{6}
ID_1	5.3	5.5	0.6	57.4

(b) Probability of infection at various doses

Dose	Mean	Median	$Q_{0.025}$	$Q_{0.975}$
0.1	3.3×10^{-5}	4.9×10^{-6}	3.6×10^{-8}	3.0×10^{-4}
1.0	2.6×10^{-3}	4.1×10^{-4}	3.9×10^{-6}	2.0×10^{-2}
10.0	0.075	0.026	4.1×10^{-4}	0.47
100.0	0.45	0.38	0.02	1.0

Table 3: Distributions of two metrics of *Trichinella* infectivity: ID_{50} and ID_1 (a), and the probability of infection at various (low) doses (b). Dose is expected number of larvae (of either sex), calculated as concentration (larvae/g) times intake of contaminated food (in g), as in eq. (1).

	P(inf)	
N	Mean	$Q_{0.975}$
1	0.072	0.83
10	0.008	0.077
100	3.9×10^{-4}	2.0×10^{-3}
10^3	9.1×10^{-6}	3.6×10^{-5}
10^4	8.8×10^{-8}	3.0×10^{-7}
10^5	2.8×10^{-9}	8.4×10^{-9}
10^6	1.3×10^{-10}	4.5×10^{-10}

Table 4: Simulated infection risk when standard surveillance of testing 1 g of meat by digestion and microscopic inspection is done for increasing numbers of samples (N), producing only negative results. The digestion test is assumed to fail with a mean probability of 0.4 (95% ci 0.016–0.915). Exposure is estimated for a single portion of 100 g undercooked meat.

Figures

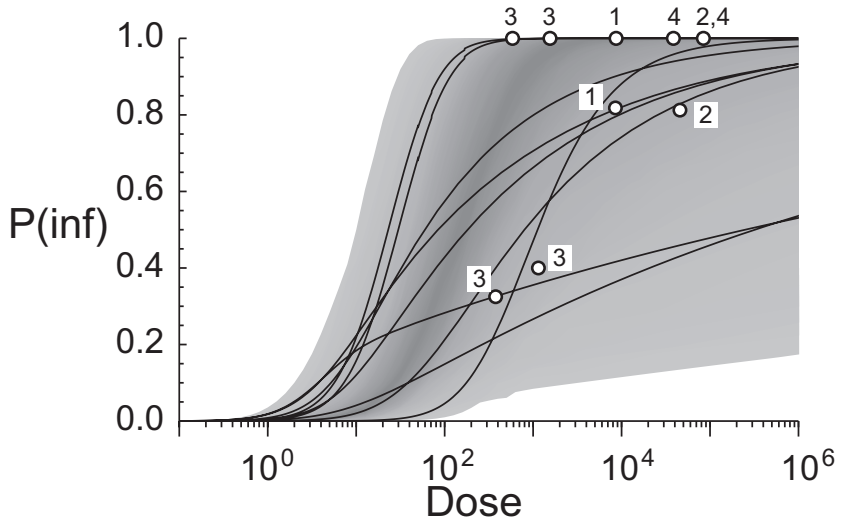

Figure 1: Outbreak based *Trichinella* dose response for infection: individual best (posterior mode) relations for each of the 10 data points from 9 outbreaks (9 curves, as Ranque et al. [5] contributes 2 different doses). Numbers indicate species: 1. *spiralis*; 2. *nativa*; 3. *britovi*; 4. *pseudospiralis*. Also shown: density graph of the predicted (generalized) probability of infection (99% interval).

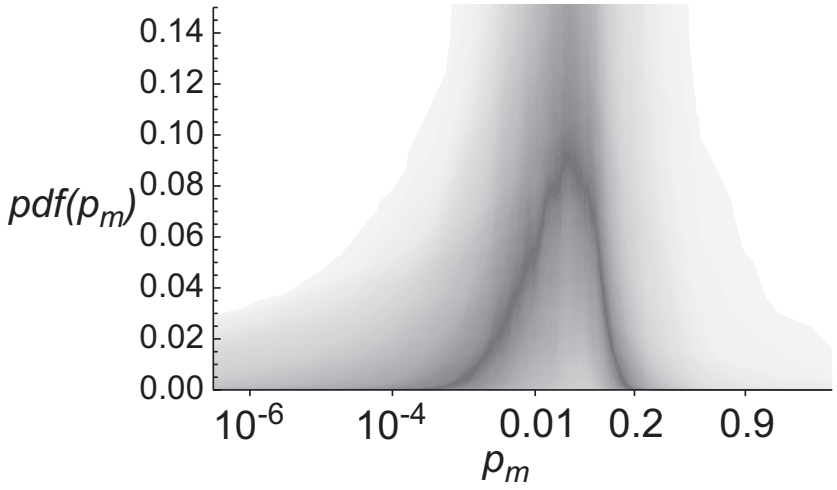

Figure 2: Density graph of the single hit probability of infection: distribution (Beta probability density) of p_m. As in Figure 1 shading corresponds to density, darkest region close to median and outer margins span a 99% interval.

Supplementary information

A The single hit model for infection

A.1 Exposure

Simplest we can assume for exposure is Poisson sample, from a suspension of strength C, sample volume V

$$\text{Prob}(n|C,V) = \frac{(CV)^n}{n!}\text{e}^{-CV} \tag{A.1}$$

Where CV is the (expected) dose, the probability of exposure

$$\text{Prob}(n \geq 1|C,V) = 1 - \text{Prob}(n = 0|C,V) = 1 - \text{e}^{-CV} \tag{A.2}$$

A.2 Infection: fixed p_m

Suppose we have a host who has ingested n pathogens, and all pathogens have equal survival probabilities p_m, then the probability that k pathogens survive is

$$\text{Prob}(k|n,p_m) = \binom{n}{k} p_m^k (1-p_m)^{n-k} \tag{A.3}$$

if survival is independent.

Infection corresponds to survival of at least 1 pathogen (a 'single hit') with probability

$$\text{Prob}(k \geq 1|n,p_m) = 1 - \text{Prob}(k = 0|n,p_m) = 1 - (1-p_m)^n \tag{A.4}$$

The marginal probability of infection therefore is

$$\text{Prob}(k \geq 1|C,V,p_m) = \sum_{n=1}^{\infty} \frac{\text{e}^{-CV}(CV)^n}{n!}\left[1-(1-p_m)^n\right] \tag{A.5}$$

which can be simplified, by first taking the sum from $n = 0$

$$P_{\text{inf}}(C,V,p_m) = 1 - \sum_{n=0}^{\infty} \frac{\text{e}^{-CV}(CV)^n}{n!}(1-p_m)^n \tag{A.6}$$

and noting that

$$\sum_{n=0}^{\infty} \frac{\text{e}^{-CV(1-p_m)}\left[CV(1-p_m)\right]^n}{n!} = 1 \tag{A.7}$$

so that

$$P_{\text{inf}}(C, V, p_m) = 1 - \mathrm{e}^{-CV}\mathrm{e}^{CV(1-p_m)} = 1 - \mathrm{e}^{-p_m CV} \tag{A.8}$$

A.3 Infection: heterogeneous p_m

In case of heterogeneity in p_m, described by a Beta pdf

$$f(p_m|\alpha, \beta) = \frac{\Gamma(\alpha+\beta)}{\Gamma(\alpha)\Gamma(\beta)} p_m^{\alpha-1}(1-p_m)^{\beta-1} \tag{A.9}$$

the marginal dose response relation for infection becomes

$$P_{\text{inf}}(C, V|\alpha, \beta) = \int_{p_m=0}^{\infty} f(p_m|\alpha, \beta)\left(1 - \mathrm{e}^{-p_m CV}\right) \tag{A.10}$$

which can be written as a (Kummer) confluent hypergeometric function

$$P_{\text{inf}}(C, V|\alpha, \beta) = {}_1F_1(\alpha, \alpha+\beta; -CV) \tag{A.11}$$

Furumoto and Mickey [23] have shown how this relation can be simplified into

$$P_{\text{inf}}(C, V|\alpha, \beta) = 1 - \left(1 + \frac{CV}{\beta}\right)^{-\alpha} \quad (\beta \gg 1; \alpha \ll \beta) \tag{A.12}$$

B Heterogeneity in the dose

B.1 Exposure, dose variable

In outbreak situations the dose often is inappropriately characterized by a simple Poisson model. Instead, we may use a Poisson-Gamma mixture to model extra–Poisson variation. The observed number is again a Poisson sample

$$\text{Prob}(n|C, V) = \frac{(C \cdot V)^n}{n!}\mathrm{e}^{-C \cdot V} \tag{A.13}$$

Where $C \cdot V$ is the (expected) dose. The concentration C now is assumed to have a Gamma density

$$g(C|\rho, \lambda) = \frac{\lambda^{-\rho}}{\Gamma(\rho)} C^{\rho-1}\mathrm{e}^{-C/\lambda} \tag{A.14}$$

with shape parameter ρ and scale parameter λ. The marginal distribution of the counts then is negative binomial

$$\text{Prob}(n|\rho,\lambda,V) = \frac{\Gamma(n+\rho)}{n!\Gamma(\rho)}\left(\frac{1}{1+\lambda V}\right)^{\rho}\left(1-\frac{1}{1+\lambda V}\right)^{n} \quad \text{(A.15)}$$

And the probability of exposure is

$$\text{Prob}(n \geq 1|\rho,\lambda,V) = 1-\text{Prob}(n=0|\rho,\lambda,V) = 1-(1+\lambda V)^{-\rho} \quad \text{(A.16)}$$

which may be written as

$$\text{Prob}(n \geq 1|\rho,\tilde{c},V) = 1-\left(1+\frac{\tilde{c}V}{\rho}\right)^{-\rho} \quad \text{(A.17)}$$

where $\tilde{c} = \lambda\rho$ is the mean concentration.

B.2 Infection, dose variable, fixed p_m

The marginal probability of infection can be found, as above

$$\text{Prob}(k \geq 1|\rho,u,p_m) = \sum_{n=1}^{\infty}\frac{\Gamma(n+\rho)}{n!\Gamma(\rho)}u^{\rho}(1-u)^{n}\left[1-(1-p_m)^{n}\right] \quad \text{(A.18)}$$

substituting $u = 1/(1+\lambda V)$.

This can be simplified by first taking the sum from $n = 0$

$$P_{\text{inf}}(\rho,u,p_m) = 1-\sum_{n=0}^{\infty}\frac{\Gamma(n+\rho)}{n!\Gamma(\rho)}u^{\rho}(1-u)^{n}(1-p_m)^{n} \quad \text{(A.19)}$$

If we note that

$$\sum_{n=0}^{\infty}\frac{\Gamma(n+\rho)}{n!\Gamma(\rho)}\left[1-(1-u)(1-p_m)\right]^{\rho}\left[(1-u)(1-p_m)\right]^{n} = 1 \quad \text{(A.20)}$$

then

$$P_{\text{inf}}(\rho,u,p_m) = 1-\left(\frac{1-(1-u)(1-p_m)}{u}\right)^{-\rho} \quad \text{(A.21)}$$

or

$$P_{\text{inf}}(\rho,\lambda,V,p_m) = 1-(1+\lambda V p_m)^{-\rho} \quad \text{(A.22)}$$

$$P_{\text{inf}}(\rho,\tilde{c},V,p_m) = 1-\left(1+\frac{\tilde{c}V}{\rho}p_m\right)^{-\rho} \quad \text{(A.23)}$$

B.3 Infection: heterogeneous p_m

In case of heterogeneity in p_m, described by a Beta pdf the marginal dose response relation for infection becomes

$$P_{\text{inf}}(\rho, \tilde{c}, V|\alpha, \beta) = \int_{p_m=0}^{\infty} f(p_m|\alpha, \beta) \left(1 - \left(1 + \frac{\tilde{c}V}{\rho} p_m\right)^{-\rho}\right) \quad \text{(A.24)}$$

which can be written as another hypergeometric function

$$P_{\text{inf}}(\rho, \tilde{c}, V|\alpha, \beta) = {}_2F_1(\alpha, \rho, \alpha + \beta; -\tilde{c}V/\rho) \quad \text{(A.25)}$$

C Sexual reproduction and infection

Suppose we have a host who has ingested of n pathogens, of whom k females (♀) and $n - k$ males (♂).

Infection can occur if and only if 1 or more ♀ pathogens and 1 or more ♂ pathogens survive. Suppose ♀ and ♂ pathogens have equal survival probabilities p_m, then the probability that 1 or more ♀ pathogens survive is

$$p_{♀} = 1 - (1 - p_m)^k \quad \text{(A.26)}$$

and the probability that 1 or more ♂ pathogens survive

$$p_{♂} = 1 - (1 - p_m)^{n-k} \quad \text{(A.27)}$$

Suppose ♀ and ♂ pathogens are present in proportions r and $1 - r$ (r is the sex ratio: the fraction ♀). Then the numbers of ♀ and ♂ pathogens are binomial

$$\text{Prob}(k♀, n - k♂|r) = \binom{n}{k} r^k (1 - r)^{n-k} \quad \text{(A.28)}$$

and the probability of infection is

$$P_{\text{inf}}(n|r) = \sum_{k=0}^{n} \binom{n}{k} r^k (1 - r)^{n-k} \left[1 - (1 - p_m)^k\right] \left[1 - (1 - p_m)^{n-k}\right] \quad \text{(A.29)}$$

which can be shown to equal

$$P_{\text{inf}}(n|r) = 1 + (1 - p_m)^n - [1 - p_m(1 - r)]^n - (1 - p_m r)^n \quad \text{(A.30)}$$

C.1 Exposure

Simplest we can assume for exposure is Poisson sample, from a suspension of strength C, sample volume V. The exposure dose response relation (de-

scribing the probability of having ingested at least 1 ♀and 1♂organism) can be written as a linear combination of three terms

$$\text{Prob}(♀ \geq 1, ♂ \geq 1|r, C, V) = 1 - \mathrm{e}^{-(1-r)CV} - \mathrm{e}^{-rCV} \tag{A.31}$$

simply by taking the terms of equation (A.30) for $p_m = 1$.

For heterogeneous exposure we can again assume a Poisson–Gamma mixture, leading to an exposure dose response

$$\text{Prob}(♀ \geq 1, ♂ \geq 1|r, \rho, \tilde{c}, V) = 1 - \left(1 + \frac{\tilde{c}V}{\rho}(1-r)\right)^{-\rho} - \left(1 + \frac{\tilde{c}V}{\rho}r\right)^{-\rho} \tag{A.32}$$

C.2 Infection, fixed p_m

For Poisson exposure and fixed "hit" probability p_m we get

$$P_{\text{inf}}(C \cdot V|p_m, r) = 1 + \mathrm{e}^{-C \cdot V p_m} - \mathrm{e}^{-C \cdot V p_m (1-r)} - \mathrm{e}^{-C \cdot V p_m r} \tag{A.33}$$

analogous to the exponential dose response relation for asexually reproducing pathogens.

For Poisson–Gamma exposure the relation is

$$P_{\text{inf}}(\rho, \tilde{c}, V|p_m, r) = 1 + \left(1 + \frac{\tilde{c}V}{\rho}p_m\right)^{-\rho} - \left(1 + \frac{\tilde{c}V}{\rho}p_m(1-r)\right)^{-\rho} - \left(1 + \frac{\tilde{c}V}{\rho}p_m r\right)^{-\rho} \tag{A.34}$$

C.3 Infection, variable p_m

The model for heterogeneous p_m can again be written as a linear combination of hypergeometric relations (see equation (A.11)).

$$P_{\text{inf}}(C \cdot V|\alpha, \beta, r) = 1 + {}_1F_1\left[\alpha, \alpha+\beta; -C \cdot V\right] - {}_1F_1\left[\alpha, \alpha+\beta; -C \cdot V(1-r)\right] - {}_1F_1\left[\alpha, \alpha+\beta; -C \cdot Vr\right] \tag{A.35}$$

In case the dose also has extra–Poisson variation, the resulting dose response relation is a combination of the functions in equation (A.25)

$$P_{\text{inf}}(\rho, \tilde{c}, V|\alpha, \beta, r) = 1 + {}_2F_1(\alpha, \rho, \alpha+\beta; -\tilde{c}V/\rho) - {}_2F_1(\alpha, \rho, \alpha+\beta; -\tilde{c}V(1-r)/\rho) - {}_2F_1(\alpha, \rho, \alpha+\beta; -\tilde{c}Vr/\rho) \tag{A.36}$$

D Hierarchical dose response model

The likelihood is binomial: for each incident K out of N subjects exposed to a dose $D = g(\rho, \tilde{c}, V)$ have been observed to be affected.

Given the hit theory dose response function a single observed attack rate may allow prediction of the dose response relation [2]. We want to incorporate multiple attack rates at various doses. However, such an approach inevitably involves an additional level of biological variation. While a different human population similar in age and health status might have similar susceptibility, a different isolate of the pathogen is likely to have completely different infectivity, if only because of a different history (different food vehicle, different previous host, ...). Therefore, analysis of data from different outbreaks requires a hierarchical model (Figure A1).

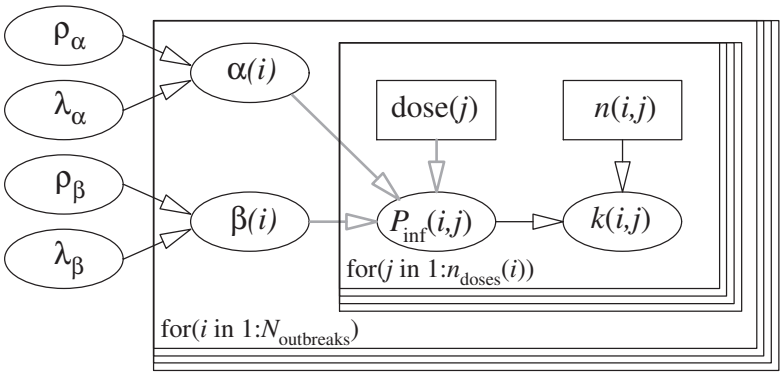

Figure A1: Two-level model for dose response assessment of several outbreaks, each with their separate pathogen isolates and possibly susceptibility distributions ($N_{\text{outbreaks}}$ = number of outbreaks; $n_{\text{doses}}(j)$ = 1 for all outbreaks except the first [5], where $n_{\text{doses}}(1) = 2$).

If there are j observations in group i and the dose response model

$$f(d|\boldsymbol{\theta}) \tag{A.37}$$

with parameter vector $\boldsymbol{\theta}$ the contribution of group i to the likelihood is

$$\ell_i(\boldsymbol{\theta}) = \prod_j [f(d_{i,j}|\boldsymbol{\theta})]^{k_{i,j}} [1 - f(d_{i,j}|\boldsymbol{\theta})]^{n_{i,j}-k_{i,j}} \tag{A.38}$$

all observations in group i share the same parameter set $\boldsymbol{\theta}$

When the joint distribution of $\boldsymbol{\theta}$ over all groups is

$$h(\boldsymbol{\theta}|\boldsymbol{\Xi}) \tag{A.39}$$

with hyperparameter vector $\boldsymbol{\Xi}$, the marginal likelihood can be written

$$\begin{aligned}\ell_i(\boldsymbol{\Xi}) &= \int_{\boldsymbol{\theta}} \ell_i(\boldsymbol{\theta}) h(\boldsymbol{\theta}|\boldsymbol{\Xi}) \mathrm{d}\boldsymbol{\theta} \\ &= \int_{\boldsymbol{\theta}} \left[f(d_{i,j}|\boldsymbol{\theta})\right]^{k_{i,j}} \left[1 - f(d_{i,j}|\boldsymbol{\theta})\right]^{n_{i,j}-k_{i,j}} h(\boldsymbol{\theta}|\boldsymbol{\Xi}) \mathrm{d}\boldsymbol{\theta} \quad \text{(A.40)}\end{aligned}$$

and the hierarchical likelihood, to be evaluated, is

$$\mathrm{L}(\boldsymbol{\Xi}) = \prod_i \ell_i(\boldsymbol{\Xi}) \quad \text{(A.41)}$$

The dose is characterized by the expected concentration of pathogens, and their variation, characterized by the Gamma shape parameter ρ. These two parameters are estimated separately using whatever information was available in the outbreak reports, usually quantiles characterizing location and spread of intake of contaminated unheated (or inadequately heated) meat.

Infectivity parameters are transformed as in [2]: since we have only one data point per outbreak, the parameters (α, β) of the Beta Poisson model are highly correlated: parameter estimation is improved by transformation to

$$\begin{aligned} u &= \alpha/(\alpha+\beta) \\ v &= {}^{10}\log(\alpha+\beta) \end{aligned} \quad \text{(A.42)}$$

so that we are estimating the mean value (u) of the Beta distribution for p_m and a quantity that is inversely related to its variance (for very large positive values of v the variance tends to zero). Further u is logit–transformed and v is log–transformed

$$\begin{aligned} w &= \log[u/(1-u)] \\ z &= \log(v) \end{aligned} \quad \text{(A.43)}$$

We use normal priors for w and z (mean ρ, standard deviation λ). Uncorrelated non–informative normal (-8,8) hyperpriors were taken for the means of w and z (rho), gamma (0.001,1000) priors were taken for the standard deviations of w and z (λ).

Posterior parameter samples have been obtained using the Metropolis-Hastings algorithm, implemented in Mathematica [2].

E Additional outbreak information

City/village name	year	nr. cases	parasite strain	asympt. cases	type of meat	cons. g/pers.	conc. larv./g	cons. abroad	meat import	animal import	local	reference
Perpignan	2001	1	NA	NA	pork	NA	NA	yes				CNR *Trichinella*
Paris	2001	1	NA	NA	NA	NA	NA	yes				CNR *Trichinella*
Quillan (Aude)	2002	4	NA	NA	Wild Boar	NA	NA				yes	CNR *Trichinella*
Villeneuve d'Entraunes (Alpes Maritimes)	2003	6	*T. britovi*	1	Wild Boar	150	3				yes	[7]
Rouen (Seine Maritime)	2004	1	*T. nativa*	0	Black Bear	300	250	yes				CNR *Trichinella*
Martigues (Bouches du Rhône)	2004	1	*T. britovi*	0	Jackal	NA	NA	yes				[24]
	2004	1	NA	NA	NA	NA	NA	yes				CNR *Trichinella*
Rouen (Seine maritime)	2005	3	NA	6	pork	NA	NA	yes				CNR *Trichinella*
Orléans (Loiret), Narbonne (Aude)	2005	17	*T. nativa*	8	Black Bear	150	300	yes	yes			[25]
Ore (31)	2006	2	NA	NA	Wild Boar	NA	NA				yes	CNR *Trichinella*
Nans les Pins (Var)	2006	3	*T. spiralis*	NA	Wild Boar	200	40					CNR *Trichinella*
Collobriëres (Var)	2006	4	*T. britovi*	6	Wild Boar	150	5–10				yes	CNR *Trichinella*

Table A1: Data from France provided by J. Dupouy–Camet (May 2007).