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Risk factors related to *Toxoplasma gondii* seroprevalence in indoor-housed Dutch dairy goats

Huifang Deng[a,c], Cecile Dam-Deisz[a], Saskia Luttikholt[b], Miriam Maas[a], Mirjam Nielen[c], Arno Swart[a], Piet Vellema[b], Joke van der Giessen[a], Marieke Opsteegh[a,*]

[a] National Institute for Public Health and the Environment, P.O. Box 1, 3720 BA Bilthoven, The Netherlands
[b] Department of Small Ruminant Health, GD Animal Health, P.O. box 9, 7400 AA Deventer, The Netherlands
[c] Faculty of Veterinary Medicine, Utrecht University, Yalelaan 7, 3584 CL Utrecht, The Netherlands

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ABSTRACT

Toxoplasma gondii can cause disease in goats, but also has impact on human health through food-borne transmission. Our aims were to determine the seroprevalence of *T. gondii* infection in indoor-housed Dutch dairy goats and to identify the risk factors related to *T. gondii* seroprevalence. Fifty-two out of ninety approached farmers with indoor-kept goats (58%) participated by answering a standardized questionnaire and contributing 32 goat blood samples each. Serum samples were tested for *T. gondii* SAG1 antibodies by ELISA and results showed that the frequency distribution of the $\log_{10}$-transformed OD-values fitted well with a binary mixture of a shifted gamma and a shifted reflected gamma distribution. The overall animal seroprevalence was 13.3% (95% CI: 11.7–14.9%), and at least one seropositive animal was found on 61.5% (95% CI: 48.3–74.7%) of the farms. To evaluate potential risk factors on herd level, three modeling strategies (Poisson, negative binomial and zero-inflated) were compared. The negative binomial model fitted the data best with the number of cats (1–4 cats: IR: 2.6, 95% CI: 1.1–6.5; >=5 cats: IR: 14.2, 95% CI: 3.9–51.1) and mean animal age (IR: 1.5, 95% CI: 1.1–2.1) related to herd positivity. In conclusion, the ELISA test was 100% sensitive and specific based on binary mixture analysis. *T. gondii* infection is prevalent in indoor housed Dutch dairy goats but at a lower overall animal level seroprevalence than outdoor farmed goats in other European countries, and cat exposure is an important risk factor.

1. Introduction

Toxoplasma gondii (*T. gondii*) is a zoonotic protozoan parasite that may cause serious disease in humans, especially when primary infection is acquired during pregnancy. In the Netherlands, the disease burden of fourteen food-borne pathogens was estimated using disability-adjusted life years. The results showed that *T. gondii* had the highest disease burden at both population and individual level (Havelaar et al., 2012).

In goats, toxoplasmosis causes abortion and stillbirth, and is thus a source of economic loss to goat farmers (Moraes et al., 2011; van Engelen et al., 2014). Moreover, goats are considered important sources of human infection, especially for ethnic groups that commonly consume goat products (Tenter et al., 2000). Tissue cysts of *T. gondii* are responsible for infections via meat (Jones and Dubey, 2012), and *T. gondii* tachyzoites can be excreted in goat's milk and can survive the raw fresh cheese-making process (Dubey et al., 2014). Infections due to the consumption of raw goat's milk have been reported (Sacks et al., 1982). There is a positive relationship between detection of antibodies against *T. gondii* in goats and presence of tissue cysts in their meat (Dubey et al., 2011). This correlation was also found between seropositivity and presence of *T. gondii* DNA in goat milk (Spišák et al., 2010). Therefore, the prevalence of antibodies in goats can be used to identify risk factors for infection but also gives an indication of the risk of infection for consumers through consuming raw goat products.

The reported percentage of *T. gondii* seropositive goats varied greatly among countries. In Europe, the seroprevalence of *T. gondii* in goats was estimated at 17% in Norway (Stormoen et al., 2012), 18.5% in north Portugal (Lopes et al., 2013), 25.1% in southern Spain (Garcia-Bocanegra et al., 2013), 30.7% in Greece (Tzanidakis et al., 2012), 52.8% in Romania (Iovu et al., 2012), and 60.6% in Italy

* Corresponding author at: National Institute for Public Health and the Environment (RIVM), Centre for Infectious Disease Control–Zoonoses and Environmental Microbiology (Cib-Z&O), A. van Leeuwenhoeklaan 9, P.O. Box 1, 3720 BA Bilthoven, The Netherlands. Fax: +31 302744434.
E-mail address: marieke.opsteegh@rivm.nl (M. Opsteegh).

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(Mancianti et al., 2013). Regional variation of seroprevalence may be caused by differences in study population, study year and climate as well as differences in serological tests and criteria of cut-off value used in the test (Tenter et al., 2000). Reported risk factors associated with *T. gondii* infection in goats are age, presence of cats, management system (i.e., extensive/intensive), source of drinking water and abortion history (Cavalcante et al., 2008; Tzanidakis et al., 2012; Gebremedhin et al., 2013; van Engelen et al., 2014).

In the Netherlands, several serological studies concerning *T. gondii* have been conducted, mainly in swine, poultry, cattle and sheep. The results showed a high seroprevalence in sheep (Opsteegh et al., 2010) compared to poultry and swine (van Knapen et al., 1982; van der Giessen et al., 2007). However, little is known concerning the seroprevalence of *T. gondii* in dairy goats. In 1998, epidemiological data on *T. gondii* infection in goats in the Netherlands were collected from ten Dutch goat farms, including three farms with a *T. gondii* abortion history. The mean seroprevalence was 47% (ranging from 5% to 90%) (Antonis et al., 1998). Since then, the number of dairy goats has nearly tripled, whereas the number of farms has decreased by 40% (Statistics Netherlands, 2015). This intensification of farming is associated with changes in farm management such as year round indoor-housing of dairy goats. In pigs and poultry, a lower seroprevalence of *T. gondii* infection was found in indoor farming systems than in outdoor farming systems (van der Giessen et al., 2007; Maksimov et al., 2011). Therefore, indoor-housed dairy goats are expected to have a low seroprevalence of *T. gondii*.

The objectives of this study were to determine the seroprevalence and to identify risk factors associated with *T. gondii* seroprevalence in Dutch indoor-housed dairy goats. A commercial indirect ELISA was used to test 1664 goat sera and serological results were analyzed using binary mixture models. Potential risk factors were evaluated by comparing Poisson, negative binomial and zero-inflated regression models.

2. Material and methods

2.1. Study population

In 2013, 451,377 goats were present in the Netherlands according to the Identification and Registration (I&R) database. Those animals were kept on 10,783 small goat farms (≤31 goats) and 546 professional goat farms (≥32 goats), of which 349 large dairy goat farms (≥50 goats). Most of the dairy goat farms use a deep litter housing system with dry straw as bedding material (Schimmer et al., 2011). This study focused on indoor-housed Dutch dairy goats on commercial farms with more than 100 goats that participated in an accreditation program for caprine arthritis encephalitis (CAE) or caseous lymphadenitis (CL), as carried out by GD Animal Health.

2.2. Data collection and sample size

A standardized questionnaire was designed to measure the exposure to putative risk factors. The questionnaire (available as Supplementary material) included 12 closed-ended questions on: presence of (young) cats, number of cats on farm, problems with mice/rats, water sources, use of automated mixer-feeder, storage of silage, history of outdoor access, replacement policy, and presence of other farm animals. Information on farm size and animal ages were collected from GD Animal Health database. To avoid effects from destocking and restocking during the Dutch Q fever epidemic (2009–2010), all farm management questions were referring to the past two years. During the data collection period (August 2013–June 2014), 90 farms submitted goats' venous blood samples to GD Animal Health as part of the accreditation programs. These 90 farmers were asked to participate in this study, to which 52 agreed. The questionnaire and informed consent form were sent by e-mail and regular mail to the 52 Dutch dairy goat farms and completed by the farm owner or manager in July–August 2014. On each farm, blood samples had been collected by the veterinarian from 44 to 149 animals of more than one year old, according to the CAE and CL monitoring sampling schemes provided by GD Animal Health. To obtain an equally precise estimate of on-farm *T. gondii* prevalence for all farms, it was decided to test the same number of animals per farm. With 52 participating farms, available ELISA tests were sufficient for 32 goats per farm, thus, 32 frozen serum samples per farm were selected and tested at the National Institute for Public Health and the Environment.

The maximum possible within-herd prevalence detectable with 32 sampled goats and at a confidence level of 95% was estimated using winepi.net. The minimum detectable incidence rate ratio (IR) between exposure to risk factors and infection (presence of antibodies to *T. gondii*) at herd level in this study with a sample size of 52 farms was determined based on: (1) the relative frequency of exposure among non-infected farms: 50%, (2) the ratio between non-infected and infected farms: 3, (3) a confidence level of 95% and allowable error of 10% (Noordhuizen et al., 2001).

2.3. Serological assay

Individual goat serum samples were tested with a commercial indirect enzyme-linked immune sorbent assay (ELISA) (ID Screen Toxoplasmosis Indirect Multi-species; ID.VET Innovative Diagnostics, France) to determine the presence of *T. gondii* specific P30 (SAG1) antibodies. All steps were carried out according to the instruction of the manufacturer. Every serum sample was tested in duplicate; plate-to-plate variation of the optical density values (OD-value) was corrected by using linear regression on the control sera tested on every plate (Opsteegh et al., 2010). A plate was retested if replicates of one control had a coefficient of variation (CV) above 20% (Reed et al., 2002), or if the mean OD-value of the positive control was lower than 0.350 and/or the ratio of the mean OD-value of the positive and negative controls was lower than 3. Individual sera were retested if the replicates showed different statuses according to the cut-off values provided by the manufacturer. Information from the farm questionnaires was blinded to the laboratory technician.

2.4. Data analysis

Information collected in the questionnaires and the serological test results were coded and entered in Microsoft Access and Microsoft Excel, and statistical analysis was performed using SPSS software version 20 and R 3.03 (IBM Corp, 2011; R Core Team, 2014).

2.4.1. Binary mixture analysis and estimation of seroprevalence

A suitable cut-off with corresponding estimates of diagnostic performance was obtained by fitting binary mixture models to the $\log_{10}$-transformed OD-values from the ELISA (Jacobson, 1998; Opsteegh et al., 2010). Visual inspection of the histogram of the data revealed flattened tails, thus suggesting that the components of the mixture may not be normally distributed. Therefore, pragmatically, all combinations of (1) normal and (2) shifted (along the *x*-axis) and optionally reflected (along the *x*-axis, when used for the positive component) gamma distributions were fitted and compared based on Akaike information criterion (AIC) values. Normal distributions and mixing parameter (prevalence) were fitted as described before (Opsteegh et al., 2010). For (reflected) gamma distributions, shape (α) and rate (β) parameters and a shift along the *x*-axis were estimated for both the negative and positive components. A cut-off value was determined at the maximum sum of

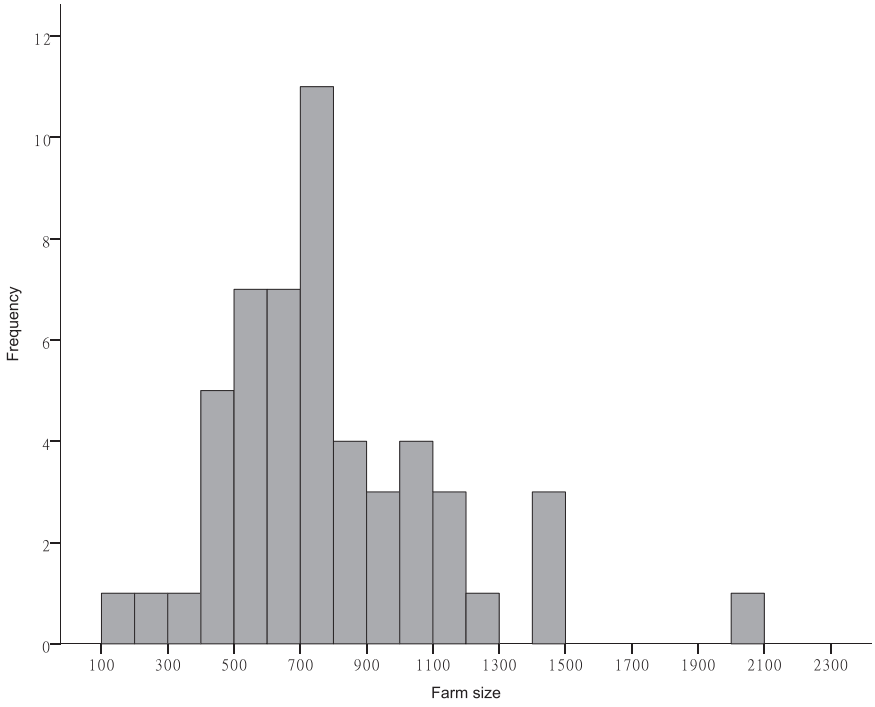

Fig. 1. Frequency distribution of farm size for 52 indoor Dutch dairy goat farms participating in *T. gondii* risk factor study.

sensitivity and specificity based on the final binary mixture model. Overall animal level seroprevalence was calculated based on the fraction of animals with an ELISA OD-value above the cut-off value provided by the manufacturer, and compared to the seroprevalence obtained from the binary mixture model. A farm was considered positive if at least one goat on the farm was positive in ELISA. Ninety-five percent confidence intervals for seroprevalence were based on normal approximation for the binomial distribution.

2.4.2. Risk factor analysis

Frequency tables of categorical variables and frequency distributions of continuous variables were constructed. Risk factors were analyzed on farm level, with mean age of the 32 selected animals per farm included as sample age. Three different modeling strategies for count data were compared: Poisson, negative binomial (NB) and zero-inflated models. Potential risk factors on farm level were analyzed by both Poisson and NB models with number of seropositive animals per farm as dependent variable and the 32 tested animals per herd as offset. In the first step, bivariable associations between each potential risk factor and the dependent variable were estimated. After testing the effect of age on seropositivity on individual animal level using a binary logistic regression model with a random herd effect, sample age was always included as a continuous variable in the farm level models to adjust the estimates. Linearity was checked by plotting residuals against sample age. All independent variables with p-value $\leq$0.2 were selected and the associations between those variables were tested with Chi-square test before being entered into the multivariable regression model in the second step. If variables were found to be correlated, decisions on inclusion of variables were made based on biological relevance, completeness of data, and the strength of relationship between the outcome and the putative risk factors. Potential confounding was determined by the change ($\geq$20%) of estimates for other independent variables before and after the factor entering the model (Dohoo et al., 2009). Missing values were excluded in the bivariable analysis, but they were coded as additional categories in the multivariable step. The model was built in a backward elimination process and biologically plausible two-way interactions between variables in the multivariable model were tested. All variables with a p-value less than 0.05 in the likelihood ratio test were kept in the model. Risk was expressed as an incidence rate ratio (IR) with 95% confidence interval (Dohoo et al., 2009). The fit of the models was checked using the Pearson goodness-of-fit test and dispersion parameter. A choice was made between the Poisson model and the NB model based on the likelihood ratio test.

Finally, because of the potential presence of excess zeros in the data, a zero-inflated model was built. First, the associations between all the variables were tested with Chi-square test. All variables except correlated ones were entered into both the count and zero parts of the zero-inflated model. Next, this full model was reduced by automatic model selection based on finite sample corrected AIC (AICc). The process was performed using MuMIn package in R (Bartoń, 2014). The fit of the Poisson or NB model and the respective zero-inflated model was compared using a Vuong test (Vuong, 1989).

3. Results

3.1. Descriptive statistics

Epidemiological data were collected from 52 out of 90 approached farms (58%) and a total number of 1664 serum samples (32 per farm) were obtained from those farms. The participating farms originated from all provinces in the Netherlands, except Groningen and Zeeland. The mean number of goats older than one year on those 52 farms was 792 (ranging from 162 to 2083) (Fig. 1), and was not different between positive (784) and negative farms (805) (independent samples t-test, $p < 0.05$). For 93% of the 1664 sampled animals, age information was available. Mean age of all individual animals was 3.3 years, and varied between 1 and 11.7 years (Fig. 2). The mean age per farm, which was used in further analyses as sample age, ranged from 1.3 to 7.2 years.

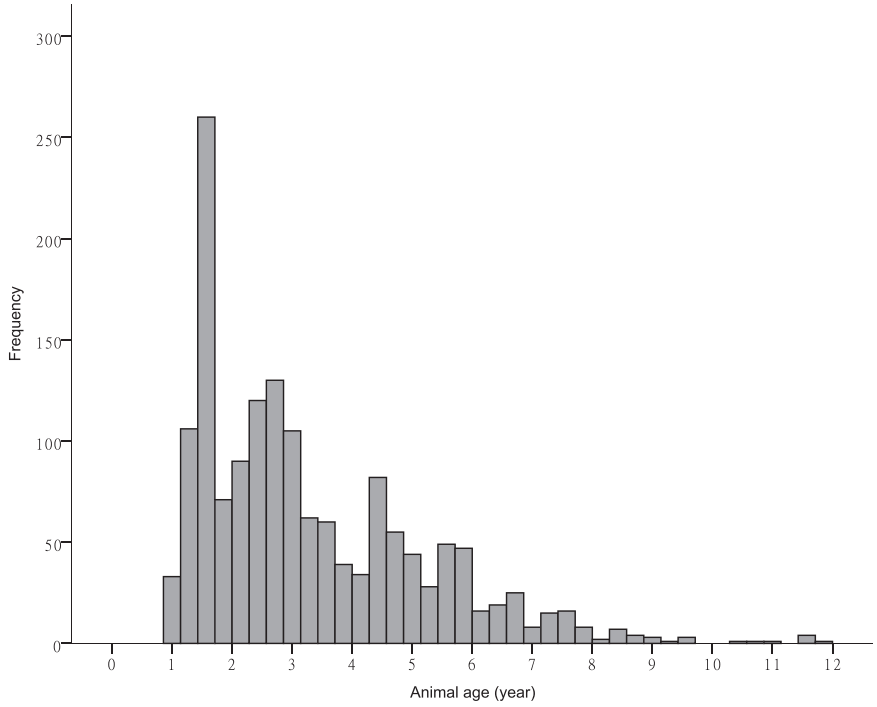

Fig. 2. Frequency distribution of age (in years) for 1664 dairy goats from 52 indoor farms participating in *T. gondii* risk factor study in the Netherlands.

3.2. Serological results

Based on the cut-off values from the commercial test, 221 out of 1664 (13.3%, 95% CI: 11.7–14.9%) dairy goats scored positive (S/P% ≥50%, which corresponded to a corrected OD-value of 0.93) and 1443 scored negative (S/P% ≤40%, which corresponded to a corrected OD-value of 0.76). Samples between 40 and 50% S/P% were defined as doubtful. On initial testing, for three animals, the duplicates showed a positive and a doubtful result. After retesting, all of the duplicates were consistently positive. Out of the 52 farms, 32 (61.5%, 95% CI: 48.3–74.7%) had one or more seropositive dairy goat(s) present and were defined as positive farms. Among the positive farms, the seroprevalence of *T. gondii* varied from 3.1% to 96.9% (Fig. 3). The odds ratio for seropositivity of *T. gondii* increased significantly with individual age (OR: 1.4, 95% CI: 1.2–1.6) based on a binary logistic regression model with animal status as outcome variable and a random herd effect ($p < 0.001$) (details not shown).

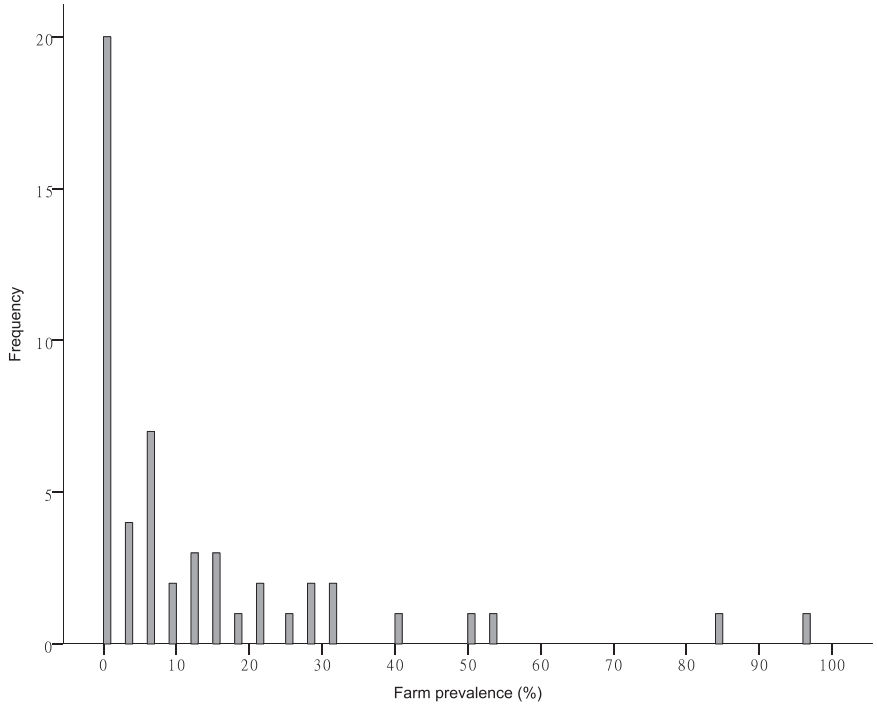

Fig. 3. Frequency distribution of within farm seroprevalence of *T. gondii* in Dutch dairy goats at indoor farms ($n = 52$ farms, with 32 animals tested per farm).

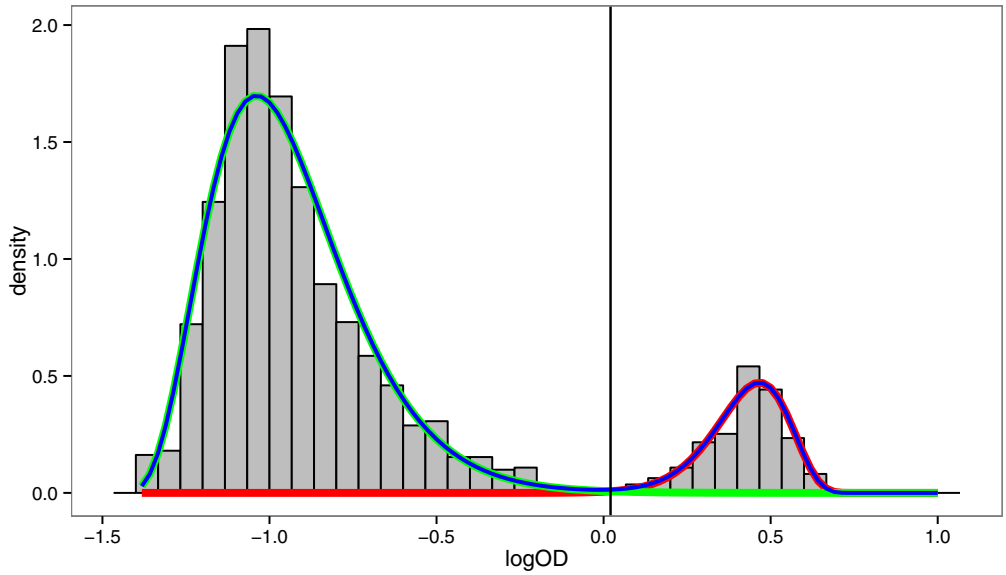

Fig. 4. Frequency distribution of $\log_{10}$-transformed OD values from 1664 Dutch dairy goats at indoor farms in *T. gondii* ELISA (bars), fitted with a mixture of a shifted gamma (left) and a shifted reflected gamma distribution (right), and cut-off value ($\log_{OD} = 0.02$) (vertical line).

The frequency distribution of the $\log_{10}$-transformed corrected OD values from the ELISA test clearly showed two separated distributions. The binary model with a mixture of a shifted gamma and a shifted reflected gamma distribution had the lowest AIC value and fitted data best (Fig. 4). The negative component is described by Gamma ($\alpha = 8.2$, $\beta = 24.4$) shifted by 0.8 along the *x*-axis, and the positive component is described by a reflected Gamma ($\alpha = 85.2$, $\beta = 10.3$) shifted by 1.5 along the *x*-axis. The overall seroprevalence was estimated at 13.2% based on this binary model. The optimum cut-off OD-value was 1.05 (the vertical line in Fig. 4), with both sensitivity and specificity estimated at 100.0%. As there were no goats with OD-values between 0.76 and 1.05, the choice of the cut-off value (manufacturer or binary mixture model) did not affect the scoring of the goats or the estimates of seroprevalence.

3.3. Risk factor analysis on herd level

The final multivariable Poisson regression model showed lack of fit (Pearson Chi-square test, $p < 0.05$) and over-dispersion (deviance/degree of freedom = 4.2, where >1 indicates over-dispersion). The likelihood ratio test suggested that the negative binomial (NB) model fitted significantly better than the Poisson

Table 1
Number of Dutch indoor dairy goat farms, on-farm prevalence and goat level seroprevalence of *T. gondii* by variable category, and sample age adjusted incidence rate ratio's (IR) with 95% confidence intervals and *p*-values (based on likelihood ratio test) for those variables in farm level bivariable negative binomial regression analysis.

Variable	Category	N[a] (%)	Farm Prev.[b] (%)	Animal Prev.[c] (%)	IR	95% CI	*p*-value[d]
Presence of cats	No	14 (26.9)	50.0	4.5	Ref.		**0.016**
	Yes	38 (73.1)	65.8	16.5	3.8	1.4–10.1	
Number of cats	0	14 (26.9)	50.0	4.2	Ref.		**0.000**
	1- 4	32 (61.5)	62.5	11.7	2.6	1.1–6.5	
	>=5	6 (11.5)	83.3	42.7	14.2	3.9–51.1	
Access of cats to goat stable	No	16 (30.8)	62.5	12.5	Ref		**0.200**
	Yes	19 (36.5)	63.2	17.9	2.1	0.7–6.7	
Presence of young cats	No	30 (57.7)	60.0	9.7	Ref.		**0.016**
	Yes	16 (30.8)	68.8	23.4	3.1	1.2–8.0	
Problems with mice or rats in the stables	No	32 (38.5)	56.2	13.2	Ref.		0.604
	Yes	20 (61.5)	70.0	13.4	1.3	0.5–3.4	
Water source	Public source only	31 (59.6)	61.3	11.1	Ref.		0.506
	Non-public source	20 (38.5)	60.0	15.3	1.4	0.6–3.3	
Use of mixer-feeder	No	26 (50.0)	50.0	10.2	Ref.		**0.122**
	Yes	26 (50.0)	73.1	16.3	2.1	0.8–5.1	
Use of silo	No, bales only	27 (51.9)	51.9	11.0	Ref.		0.262
	Yes	23 (44.2)	73.9	17.0	1.7	0.7–4.0	
Outdoor access	No	43 (82.7)	58.1	14.6	Ref.		0.337
	Yes	9 (17.3)	77.8	6.9	0.5	0.2–1.8	
Replacement policy	Closed farm	18 (34.6)	55.6	7.1	Ref.		0.240
	Only male goats introduced	24 (46.2)	62.5	18.6	2.4	0.9–6.4	
	Both male and female goats introduced	10 (19.2)	70.0	11.6	1.6	0.5–5.4	
Presence of other farm animals	No	23 (44.2)	56.5	8.0	Ref.		**0.137**
	Yes	28 (53.8)	64.3	17.0	2.0	0.8–4.9	

[a] Total number per variable may vary because of missing values.
[b] Farm Prev. stands for the prevalence of farms with at least one seropositive animal in each category.
[c] Animal Prev. stands for the prevalence of seropositive animals in each category.
[d] *p*-values ≤0.20 are presented bold; these factors were considered for inclusion in the multivariable model.

Table 2
Incidence rate ratios (IR) for variables associated ($p < 0.05$ in likelihood ratio test) with *Toxoplasma gondii* seropositivity at indoor Dutch dairy goat farms ($n = 52$) in multivariable negative binomial regression analysis.

Variable	Category	*N* (%)	IR	95% CI	*p*-value
Number of cats	0	14 (26.9)	Ref.		0.000
	1–4	32 (61.5)	2.6	1.1–6.5	
	>=5	6 (11.5)	14.2	3.9–51.1	
Sample age	Continuous	NA	1.5	1.1–2.1	0.011

NA: not applicable.

model ($p < 0.001$). In addition, a zero-inflated NB model was built, but the Vuong test indicated superior fit of the NB model ($p < 0.001$). Six variables with a $p \leq 0.2$ were found in the bivariable NB regression analysis (Table 1). However, number of cats was significantly correlated with presence of cats, access of cats to the stable and presence of young cats (Pearson Chi-Square, $p < 0.05$), therefore only number of cats, use of mixer-feeder, presence of other farm animals and sample age were entered into the multivariable NB regression model. The final model maintained only two predictors: number of cats (1–4 cat: IR: 2.6, 95% CI: 1.1–6.5; ≥5 cats: IR: 14.2, 95% CI: 3.9–51.1) and sample age (IR: 1.5, 95% CI: 1.1–2.1) (Table 2). The NB model fitted the data well (Pearson Chi-square test, $p < 0.05$) with a dispersion coefficient of 1.4 (95% CI: 0.8–2.5).

4. Discussion

A commercial ELISA test (ID Screen Toxoplasmosis Indirect Multi-species; ID.VET Innovative Diagnostics, France) was used to determine the presence of *T. gondii* specific P30 (SAG1) antibodies in goat serum samples. This is a multispecies ELISA, with a sensitivity and specificity of 86% and 99% respectively in experimentally infected swine (Bokken et al., 2012), and a sensitivity between 95% and 97% and a specificity of 97% in Romanian household cats (Györke et al., 2011). No information on sensitivity and specificity of the assay for use with goat sera was available. Therefore, the assay was evaluated by binary mixture analysis on the frequency distribution of observed $\log_{10}$-transformed OD-values. The histogram showed two clearly separated components with an apparent right-skew for the seronegative component and left-skew for the seropositive component. When fitted with a binary mixture model, a cut-off value with sensitivity and specificity both at 100% could be found. This indicates that the serological assay has a strong discriminatory power for classifying dairy goats as positive or negative for *T. gondii* antibodies. However, external validation based on the results with a different assay would be valuable. The skewness of the distributions is confirmed by the superior fit of the combination of a shifted gamma and a shifted reflected gamma distribution and may have resulted from OD measurements outside the linear relation between OD and antibody concentration. Overall animal level seroprevalence of *T. gondii* infection was estimated at 13.3% (95% CI: 11.7–14.9%). This seroprevalence is much lower than previously reported in goats (47%) and adult sheep (48.1%) in the Netherlands (Antonis et al., 1998; Opsteegh et al., 2010), and also low compared to results in goats in many other countries, ranging from 30.7% up to 77% (Tenter et al., 2000; Iovu et al., 2012; Tzanidakis et al., 2012; Mancianti et al., 2013). Another interesting finding was that on 38% of the 52 farms all tested goats were seronegative, whereas seropositive animals were present on all ten investigated farms (including three with a *T. gondii* abortion history) in 1998 (Antonis et al., 1998). Selection bias is expected to be limited in our study, as the majority of large dairy goat farms in the Netherlands participate in the CAE and CL monitoring programs from GD Animal Health, and the 90 farms were approached because they were planned to submit goat blood samples to the institute during the study period. However, it cannot be ruled out that the farmer's willingness to participate is influenced by, for example, a known *T. gondii* abortion history at the farm. In conclusion, indoor housing appears to reduce exposure of goats to *T. gondii* but not as much as has been shown for pigs and poultry (van der Giessen et al., 2007). This was expected as goat housing is less confined than pig or poultry housing, with e.g. natural ventilation and introduction of bedding, silage and roughage into the stable.

A short standardized questionnaire was used for farm level risk factors. Potential risk factors without anticipated variation between Dutch herds, i.e. the bedding material, type of housing and feeding system, were excluded from the questionnaire. To evaluate the potential risk factors, three modeling strategies were compared. There was clustering in the dataset (Fig. 3) and the Poisson model demonstrated over-dispersion. Therefore, a NB model was built, and the dispersion coefficient in the NB model was significantly larger than zero, indicating this model was more appropriate than a Poisson model. To evaluate the presence of excess zeros, a zero-inflated NB model was additionally built, but the Vuong test indicated that the NB model fitted better. Number of cats remained a significant predictor in the NB model for the number of seropositive goats on the farm, with an increased positive association for farms with 1–4 cats to farms that had ≥5 cats. This finding is consistent with previous reports (Cavalcante et al., 2008; Neto et al., 2008). Felids are the only known definitive host of *T. gondii,* and primary infected cats shed millions of oocysts in the environment (Tenter et al., 2000). The association with the presence of cats is therefore assumed to indicate a causal relationship, and limiting the number of cats on goat farms is expected to reduce *T. gondii* infections in goats. In this study, other variables that could potentially increase exposure to cat shed oocysts (e.g., access of cats to the stable, water source, use of silo, use of mixer-feeder, and history of outdoor access) did not show a statistically significant association with *T. gondii* seropositivity. This may have been due to a lack of power. Fifty-two farms participated and 32 animals per farm were tested. With 32 animals tested per farm of 792 goats on average, farms can be misclassified as negative and the maximum possible prevalence at negative farms is 8.8%. This type of misclassification is unlikely to depend on the exposure to risk factors, but can reduce the risk estimates. In addition, with 52 farms, the minimum detectable IR between exposure to risk factors and presence of *T. gondii* at herd level is estimated at 4, indicating that predictors with a weaker effect were unlikely to be identified as statistically significant in this study.

In conclusion, the serological assay used in this study was suitable for the detection of *T. gondii* antibodies in dairy goats. Indoor-housed Dutch dairy goats were not free from *T. gondii* infection, but the seroprevalence of these indoor kept dairy goats was lower than the published findings in outdoor goats in other European countries. Moreover, the number of cats on the farm was clearly associated with the number of seropositive goats. Since the overall animal level seroprevalence was 13.3% and a positive relationship between detection of antibodies against *T. gondii* and presence of tissue cysts in meat was found in this species, goats could be a source of *T. gondii* infection for humans. Goat meat and milk should be given a sufficient heat treatment to kill the parasites before consumption. Limiting the presence of cats on the goat farms is expected to reduce the prevalence of *T. gondii* infected goats.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.prevetmed.2015.12.016.

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