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Research Paper

Cats and Goat Whey Associated with *Toxoplasma gondii* Infection in Pigs

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ABSTRACT

In organic livestock production systems, farm-management factors are thought to play an important role in the on-farm prevalence of *Toxoplasma gondii*. Serological results and the results of an HACCP analysis were combined to determine important risk factors for the prevalence of this protozoan parasite. Mathematical analysis demonstrated that feeding goat whey to pigs and the presence of a high number of cats were positively correlated to *T. gondii* seroprevalence in pigs. Not covering roughage and the farmers' assumption that pigs can come into contact with cat feces also showed a positive relationship. In order to decrease the risk of *T. gondii* infecting their pigs, farmers should limit the access and number of cats on their farms and refrain from feeding goat whey to their pigs. Key Words: Toxoplasmosis—Pigs—Zoonosis. Vector-Borne Zoonotic Dis. 6, 266–274.

INTRODUCTION

Preventing contact between farm animals and zoonotic pathogens is important in both conventional agriculture and organic animal husbandry. This is difficult in organic production systems since the animals are allowed outdoors and thus have easy access to potential sources of hazardous bacteria and/or parasites. The protozoan parasite *Toxoplasma gondii* is a good example of such a microbial food-safety hazard. It causes toxoplasmosis, the most prevalent parasitic zoonotic disease in the world (Tenter et al. 2000), which can result in substantial health disorders in humans, including mental retardation, encephalitis, and blindness.

Consumption of raw or undercooked meat (pig, goat, sheep, or poultry) is known to be an important risk factor for humans in contracting toxoplasmosis (Cook et al. 2000). *T. gondii* parasites remain viable in cysts if the meat is not well prepared and can thus cause infections in humans. Unfortunately, treatment of toxoplasmosis is difficult because available drugs are not always effective (Gilbert et al. 2003, Stanford et al. 2003). Prevention of the parasite's presence at the farm level, therefore, is one of the strategies in the battle against toxoplasmosis (Kijlstra et al. 2004a).

T. gondii has a complex life cycle. Cats function as definitive hosts during one stage of *T. gondii*'s complex life cycle and transmit the parasite to the environment through defecation

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(Dubey et al. 1995). In fact, an infected cat can shed millions of *T. gondii* oocysts via its feces that, after sporulation and upon intake by intermediate hosts such as rodents, can infect other species. Farm animals, for example, can become infected by ingesting the tissues of intermediate hosts or by consuming soil, water, or feed that is contaminated with oocysts. Currently, The Netherlands has a population of 3.3 million cats in a total area of 35,054 km^2 (i.e., 94 cats/km^2). This large feline population and the Dutch climate (moist summers and mild winters, similar to that of other West European countries) offer conditions conducive to *T. gondii* growth.

The number of Dutch slaughter pigs infected with *T. gondii* decreased rapidly from over 50% seroprevalence in 1969 to 0% seroprevalence in 2001 (Kijlstra et al. 2004a), because of intensive farm-management practices whereby the animals were confined indoors (Van Knapen et al. 1995). During the last decade, however, consumer demands for farming practices that offer better animal welfare have led to an increase in organic animal production, where pigs have outdoor access and straw bedding, and are fed roughage. As suggested by the results of a theoretical Hazard Analysis and Critical Control Points (HACCP) analysis (Kijlstra et al. 2004b), these circumstances could lead to an increased risk of pigs contracting the parasite (Kijlstra et al. 2004a,b). In order to test the results of that analysis, we conducted a questionnaire-based survey and serological testing in 2004 to determine the prevalence of *T. gondii* in pigs on 36 organic pig farms in The Netherlands.

METHODS

In total, 2796 pigs from 41 organic pig farms were tested for *T. gondii* infection at the slaughter line. Between one and seven batches of pigs (each batch consisting of 15–28 animals) per farm were tested from June to September 2004. The total number of animals tested varied from 15 to 140 pigs per farm. A competition enzyme-linked immunosorbent assay (ELISA) with a peroxidase-labeled monoclonal IgM anti-SAG1 antibody (Lind et al. 1997) was used for the serological detection of *T. gondii* infection.

During the same period, a questionnaire-based survey was conducted among 36 of the participating farmers (on site). These farmers already knew about the study's background and the potential consequences *T. gondii* infection has on food safety. The goal of the questionnaire was not primarily to analyze the risk of infection, but to function as a tool for the farmers to limit the *T. gondii* infection rate. The questionnaire consisted of nine questions (including sub-questions; see Appendix) about cats, feed, farm management, and piglet supply, because these aspects had been identified as possible risk factors during the earlier HACCP methodology study (Kijlstra et al. 2004b).

The goal of this study was to combine the serological results with the results of the questionnaire in a mathematical model in order to verify the important risk factors for *T. gondii* infection. First, a bi-plot analysis with Genstat 6.0 software (Rothemsted Research, Harpenden, UK) was used to determine the coherence between the different variables in the questionnaire. A bi-plot (Gabriel 1971) is a graphical representation of the relationships between *n* individuals and *p* variates. If these variates are arranged as a matrix X(n′p), the singular value decomposition of X (X=USV¢) is used to express the least-squares approximation to X in two dimensions in the form X2=AB¢, where X2 is (n′2); A (n′2) and B (p′2) are given in the first two columns of (USr) and (VS(1-r)), respectively. When strongly correlated variables were found, we preferred those with a low non-response (unfortunately not all questions were answered by all farmers) and an objective nature (some questions were subjective, e.g., "are only older cats present?," where "older" is of course a subjective term).

The preliminary base model was then expanded using step-by-step regression with significant variables that had a lower non-response or were less objective. Variables that had a low non-response and were objective, yet not significant, were eliminated from the model. Thus, a number of significant explanatory variables remained: x1 (pigs not fed goat whey vs. pigs fed goat whey), x2 (low vs. high number of cats present), and x3 (not covering vs. covering roughage). Explanatory variables are those variables that can be used in a relationship to explain or predict changes in the values of another variable (in our case *T. gondii* infection).

Finally, the number of *T. gondii*–seropositive pigs was analyzed for the selected explanatory variables. A logistic model (Generalized Linear Mixed Model) was used both during the selection phase of explanatory variables and in the final model. A dispersion parameter was also estimated because of overdispersion in the data (McCullagh et al. 1989).

The model describes the relationship between the risk of infection (p, where $0 < p < 1$) of pigs tested in the slaughterhouse and the explanatory variables. We used the logit-link function:

$$\text{Logit (p)} = \ln (p/(1-p)) = b0 + b1x1 + b2x2 + b3x3 + b4x4$$

where: b0 = mean (on logit-scale) of the combination of all factors in the lowest class; x1 = pigs not fed goat whey (x1=0) vs. pigs fed goat whey (x1=1); x2 = having less than 3 cats (x2=0) vs. more than 3 cats (x2=1); x3 = not covering roughage (x3=0) vs. covering roughage (x3=1); x4 = assuming contact between pigs and cat feces as impossible (x4=0) vs. assuming it possible (x4=1); b1 = effect (on logit-scale) of not feeding goat whey vs. feeding goat whey; b2 = effect (on logit-scale) of having less than 3 cats vs. having more than 3 cats; b3 = effect (on logit-scale) of not covering roughage vs. covering roughage; b4 = effect (on logit-scale) of assuming contact with cat feces as impossible vs. assuming it possible.

A model assumption is that the variance of the observed number of infected pigs of a hypothetical farm Y can be described by the variance (YIp) = φnp(1-p). In this formula, *n* is the total number of pigs provided by farm Y. Because of missing answers with regard to variables x2 and x3, the final model was based on data from 26 of 36 farms.

RESULTS

Of the 2796 samples tested, 85 (3%) were positive for *T. gondii*. On the farm level, 19 of the 41 (46%) farms were *T. gondii*–negative, while 22 (54%) were positive. There was only one seropositive pig, however, on six of these farms (14%). Forty-one of the 148 batches that arrived at the slaughterhouse contained at least one seropositive pig (27.7%; Table 1).

Table 2 presents a selection of replies to the questions that offer insight into the current farm-management practices of organic pig farmers. These questions were selected because of their low level of non-response and their simple interpretation. For mathematical (bi-plot) analysis, all questions that were answered with "yes" were given a value of 0, while all questions answered with "no" were given a value of 1.

Figure 1 shows a graphic display of the coherence (bi-plot) between the answers to questions concerning cats. This bi-plot analysis was performed to determine the correlations between all variables and to optimize the model by selecting variables that had a low non-response and were objective.

The variables are represented by vectors in the bi-plots and the direction and length of the vectors indicate how each variable contributed to the two principal components in the plot. The vectors are situated close together if the variables were positively correlated, point in opposite directions if they were negatively correlated, and lie perpendicular to each other if they were not correlated. The observations in this plot are represented by dots and their locations indicate the score of each observation for the two principal components in the plot.

As noted above, only 36 of the 41 farmers received the questionnaire (Table 1). The results of 26 questionnaires were selected for the final model, because of missing answers with regard to variables x2 and x3. Because not all questions concerning cats were answered by all farmers, the main data set had to be divided into subsets. Figure 1 presents the subset (17 farms) in which all cat questions were answered.

Answers to questions 1.2, 1.3, and 6.4 (for questions, see Appendix) showed a strong positive correlation to each other. Answers to questions 1, 1.4, and 1.5 were positively correlated to each other, but negatively to that of question 1.1. Questions 1 (Is contact between pigs and cat feces possible?) and 1.2 (Are there <3 cats present?) were selected as in-

TABLE 1. SEROLOGICAL DATA OF THE FARMS

Farm	*No. of batches*	*No. of batches positive*	*Total no. of pigs*	*No. of pigs positive*	*Percentage of pigs positive*
A	1	1	20	1	5
B	4	1	66	1	1.5
C	4	1	80	1	1.3
D	4	1	51	1	2
E	5	1	95	1	1.1
F	4	1	75	1	1.3
G	5	5	95	26	27.4
H	1	1	15	14	93.3
I	2	0	35	0	0
J	6	0	110	0	0
K	3	0	50	0	0
L	4	2	75	2	2.7
M	4	0	75	0	0
N	4	2	75	2	2.7
O	4	0	70	0	0
P	6	2	115	3	2.6
Q	5	0	78	0	0
R	5	3	95	3	3.2
S	2	2	44	3	6.8
T	4	0	70	0	0
U	4	1	75	2	2.7
V	3	0	55	0	0
W	5	0	95	0	0
X	5	0	98	0	0
Y	4	0	75	0	0
Z	3	3	50	4	8.0
AA	6	2	115	5	4.3
AB	3	2	60	3	5.0
AC	7	2	140	2	1.4
AD	1	0	20	0	0
AE	2	0	40	0	0
AF	4	2	80	3	3.8
AG	4	2	80	2	2.5
AH	3	0	60	0	0
AI	4	0	76	0	0
AJ	3	1	60	1	1.7
AK	3	2	60	2	3.3
AL	4	0	80	0	0
AM	1	0	20	0	0
AN	1	1	20	2	10
AO	2	0	48	0	0
Total	148	41	2796	85	3.0

dependent cat variables for the final stage of analysis.

Figure 2 provides a graphic display of coherence (bi-plot) between the answers to questions on feeding aspects and the selected variables concerning cats. The answer to question 1.2 was negatively correlated to that of question 5, and positively to those of questions 3,

TABLE 2. SUMMARY OF REPLIES TO SOME QUESTIONS ON THE QUESTIONNAIRE

Factor	*Question no.*	*Yes, %*	*No, %*	*No. of replies*
Contact with cat feces is assumed possible	1	61.1	39.9	36
>3 cats present on the farm	1.2	33.3	66.7	31
Goat whey is fed to pigs	5	8.3	91.6	36
Rodent control is practiced	6.5	100	0	36
Roughage is covered	6.7	87.5	12.5	32
Feed manufacturer guarantees heading of >70°C	8.1	17.1	82.9	35

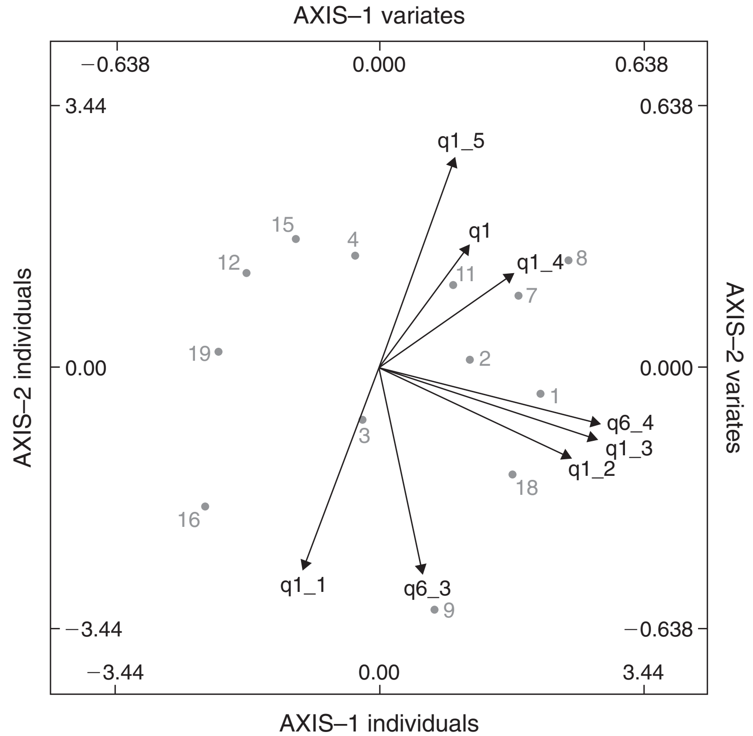

FIG. 1. Coherence of questions concerning cats (bi-plot, question numbers are preceded by the letter q; for example, q1_5 refers to question 1.5) from the subset of 17 farms. Observations (farm results) are represented by points in this plot. Some have exactly the same location in the plot and overlap each other.

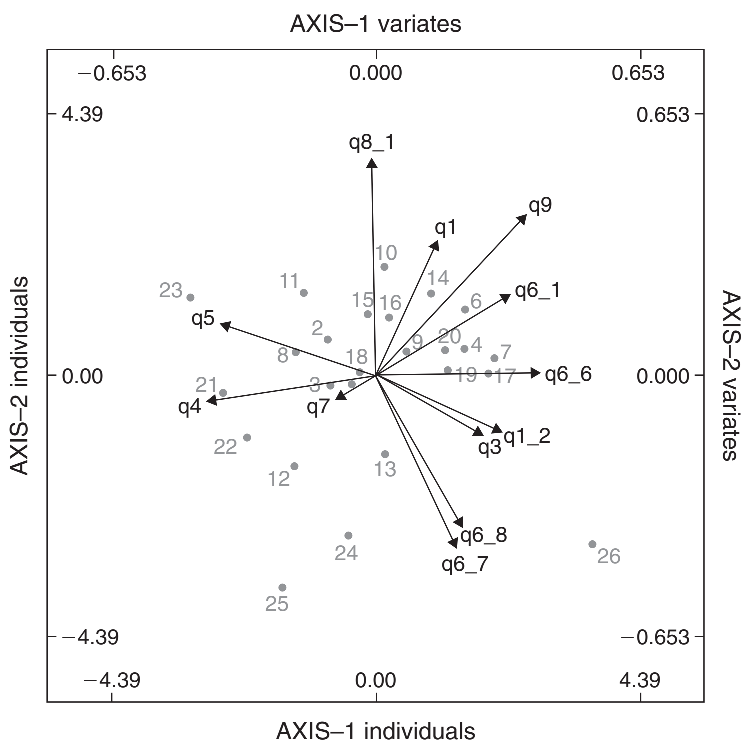

FIG. 2. Coherence of questions concerning feeding and selected cat variables (bi-plot, question numbers are preceded by letter q) from the main data set (26 farms).

TABLE 3. ODDS RATIO ESTIMATES OF THE SELECTED EXPLANATORY VARIABLES

Parameter	*Factor*	*Question no.*	*Odds ratio*	*Significance*
b1	Goat whey is fed to pigs	5	6.67	<0.01
b2	>3 cats present on the farm	1.2	2.07	0.15
b3	Roughage not covered	6.7	13.45	<0.001
b4	Contact with cat feces assumed possible	1	4.55	<0.01

6.6, 6.7, and 6.8. Answers to questions 6.7 and 6.8 were positively correlated to each other and negatively to those of questions 8.1 and 1. The answer to question 7 was positively correlated to questions 4 and 5, and negatively to those of questions 6.1, 9, and 1. Interpretation of the biplot models is complicated because of the manner in which the questions were formulated in the questionnaire. The horizontal axis in Figure 2 represents the effects of goat whey, drinking basins, and cats. The farms on the left side of the plot did not feed goat whey to their pigs (q5), did not use floating drinking basins (q4), and did not have more than three cats (q1.2), while the farms on the right did. Thus, it can be deduced from Figure 2 that "feeding goat whey" and "having more than three cats" are positively correlated to each other. All these variables were selected for the final model. Odds ratio estimates of the selected explanatory variables are displayed in Table 3.

Toxoplasma gondii infection levels varied between 0% and 10% on all but two farms: farms G and H had infection levels of 27% and 93%, respectively. Animals from farm G were tested in five consecutive batches consisting of 15–20 animals each. Of these, two to eight animals per batch were seropositive. Farm H (number 26 in Fig. 2) was a smaller farm and only delivered one batch of 15 pigs, 14 of which were infected. The estimated effects of the factors "not covering roughage" (parameter b3) and "assessing contact between pigs and cat manure as impossible" (b4) can be highly attributed to these farms. As a result, we also analyzed our data excluding these two farms (Table 4).

DISCUSSION

The use of odds ratios is advantageous because one can then speak about the increase in risks of a certain situation compared to a reference situation. Using observational data, the results of the present study showed a relationship between *Toxoplasma gondii* in pigs at the slaughter line and several farm-management aspects. We could conclude that the number of cats present on farm and feeding pigs goat whey were both positively related to the seroprevalence of *T. gondii* in pigs. The first relationship did not come as a surprise since earlier studies recognized cats as an important risk factor (Dubey et al. 1995, Meerburg et al. 2004). To our knowledge, however, the second relationship (transfer of toxoplasmosis to pigs through goat whey consumption) has not been previously reported, even though drinking milk has been implicated in the transfer of toxoplasmosis. Studies by Riemann et al. (1975) and Sacks et al. (1982) showed an association between acute toxoplasmosis in humans to the consumption of unpasteurized goat's milk and *T. gondii* tachyzoites have been found in the milk of sheep, goats, and cows (Tenter et al. 2000). However, the level of infectivity of tachyzoites remains a subject of discussion: some studies claim that tachyzoites are killed

TABLE 4. ODDS RATIO ESTIMATES EXCLUDING DATA FROM THE TWO EXTREME FARMS

Parameter	*Factor*	*Question no.*	*Odds ratio*	*Significance*
b1	Goat whey is fed to pigs	5	3.57	<0.01
b2	>3 cats present on the farm	1.2	3.24	0.04

by gastric acid (Jacobs et al., 1960). Dubey (1986) claims that, in the case of children, proteolytic enzymes in the gastrointestinal tract are not working as effectively as in adults and tachyzoites can survive the passage through the stomach. Definitive proof of transfer of toxoplasma infection via milk has been shown in mice (Pettersen 1984).

A theoretical HACCP analysis of *T. gondii* infection at organic pig production facilities via goat whey (Kijlstra et al. 2004b) revealed that the chance of contamination (whether the described risk occurs on farm) may be relatively low, but the severity (number of pigs affected by *Toxoplasma* when the risk becomes manifest) is high. Although transfer of toxoplasma infection via goat whey is a quite likely phenomenon, the actual evidence showing transfer of tachyzoites via goat whey is still lacking.

Not covering roughage and farmers assuming a possible contact between pigs and cat feces also seem to have a certain influence on the seroprevalence of *T. gondii* in pigs, although this can mostly be attributed to the two "extreme" farms G and H where *T. gondii* seroprevalence reached 27% and 93%, respectively. Even though farm H was a small farm, the seroprevalence was high because the farmer assumed possible contact between pigs and cat feces, more than three cats were present, roughage was not covered, and pigs drank goat whey. Interestingly, farm G produced a continuous level of infection. This continuity may be related to poor rodent control since another study by our group (Meerburg et al. 2006) reported numerous rodents at this particular farm. Because other farm-management factors may be involved, more research is necessary to find the sources of infection at this particular farm.

Pigs drinking goat whey (a byproduct of cheese-making, a process in which unpasteurized milk is transformed into cheese) had a strong coherence with the keeping of many (three or more) cats (Fig. 2). Not covering roughage was strongly correlated to failure to repel cats and pest animals from hay, straw, roughage, and feedstuff. Although the latter (e.g., failure to avoid cats) may not be a very suitable question (6.8) because of its diverse nature, it does provide good insight into a farm's hygiene status. Further, the effect of assuming no contact between pigs and cat feces was strongly correlated to the fattening of a farm's own piglets and with neglecting to question feed manufacturers about guarantees concerning feed-heating temperatures.

An earlier study pointed out that poor rodent control is a risk factor for *T. gondii* on pig farms (Weigel et al. 1995). The effect of pest control (including rodents), however, could not be directly estimated in this study, because all 36 farmers answered question 6.5 positively, i.e., they use some form of pest control. Since it remains uncertain to what extent this control is indeed performed, one cannot claim that this factor is unimportant for the occurrence of *T. gondii* in pigs. Moreover, the farmers were aware of this risk factor so their answer could be biased.

In order to guarantee safe organic pork, more emphasis should be placed on the importance of certain farm-management factors. For example, organic farmers can easily switch from feeding goat whey (or sheep whey) to providing other products that have the same nutritional values, but are heated during the production process. In our opinion, feeding pigs unpasteurized goat or sheep whey should be discouraged until further scientific evidence concerning the risk of transfer becomes available. Further, although it is impossible to prevent all contact between pigs and cats/rodents in an organic setting, it is possible to restrict the frequency. Pest-proofing farm buildings and removing access to feed, water, and shelter will help limit not only the presence of cats, but also the number of pest animals (Meerburg et al. 2004). It will also lower the number of birds, thus further minimizing the amount of prey for cats. Although it is not recommendable from a food-safety point of view, organic farmers frequently use cats as rodent exterminators instead of applying rodenticides (Kijlstra et al. 2004a). These agents are relatively easy to use, but may eliminate non-target species, be cruel in their action, and weaken rodents, thus facilitating their consumption by, for example, pigs and increasing the risk of *T. gondii* infection. Better options include the use of traps or installing perches or nest boxes to stimulate the presence of birds of prey (Meerburg et al. 2004).

Another option to limit the risk caused by the presence of cats is the administration of a feline *T. gondii* vaccine. The use of this vaccine was found to reduce *T. gondii* incidence in pigs (Mateus-Pinilla et al. 1999). Vaccination, however, is only possible with the farmers' own cats, not with all other cats in the neighborhood, as they have other owners. In order to overcome this problem, we recommend the nationwide integration of *Toxoplasma* vaccination into the standard kitten vaccination schedules. Until this is realized, farmers should reduce the number of cats on their farms and limit their access to the farm premises in order to decrease the risk of *T. gondii* infection. Moreover, farmers should be aware of the risks of feeding their pigs "animal products" such as whey.

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APPENDIX

Questions concerning farm management and Toxoplasma gondii

1. Do you assume that pigs can come into contact with cat feces (e.g., in the pens or in the outdoor area)?

1.1. Do you bar cats from the outdoor area?
1.2. Are fewer than three cats present on the farm?
1.3. Are only older cats present?
1.4. Are the female cats sterilized?
1.5. Do you have a male cat that defends the outdoor area?

2. Are the piglets or pigs fed compost?

If so:

2.1. Do you plan not to feed compost anymore because of the possible transmission of various pathogens? (yes = no more compost will be supplied)

3. Do the pigs have access to water from ditches?

If so:

3.1. In the future will you prevent pigs from having access to ditch water because of potential contamination risk? (yes = it will be prevented in the future)

4. Do you use floating drinking basins?

If so:

4.1. Are your floating drinking basins closed to prevent contamination?

5. Do you feed goat whey to your pigs?

If so:

5.1. Do you plan to stop feeding goat whey to your pigs?

6. Do you feed cut products, roughage, straw, or hay to your pigs?

If so:

6.1. Is it correct that cats do not have access to feed or feeding equipment (yes = yes, that is correct)?
6.2. Do you repel pest animal access to feed or feeding equipment?
6.3. Do you acquire your roughage from farms with only a few cats?
6.4. Do you only have older cats (is repetitive if question 1 is answered as "yes")
6.5. Do you apply pest control?
6.6. Do you apply pest control by trapping?
6.7. Do you cover your roughage?
6.8. Do you always prevent contact between cats/pest animals and the cut products, roughage, hay, or straw on your farm?

7. Do cats, birds, or pest animals have access to the pelleted feed trajectory (silo, feed pipes, feed cart, or trough)?

If so:

7.1. Is it correct that cats do not have access to feed or feeding equipment (yes = yes, that is correct)?

7.2. Do you apply pest control by trapping (is repetitive if question 6 is answered as "yes")?

7.3. Do you check the feed and feeding equipment with regard to cleanliness (free from manure, etc.)?

8. Do you provide pelleted feed to your pigs?

If so:

8.1. Do you ask feed manufacturers for a guarantee that feed is heated to temperatures above 70° Celsius?

9. Do you obtain piglets of other farms?

If so:

9.1 Do you obtain piglets of farms that guarantee they are free of *Toxoplasma gondii*?

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