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A Bayesian approach for estimating values for prevalence and diagnostic test characteristics of porcine cysticercosis

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Abstract

Several diagnostic techniques are used to estimate the prevalence of the zoonotic tapeworm *Taenia solium* in pigs, but none of these tests are perfect, making interpretation of results difficult. A Bayesian approach was used to estimate values for the prevalence and diagnostic test characteristic of porcine cysticercosis by combining results of four imperfect tests. Village pigs ($N = 868$), slaughtered in Lusaka (Zambia), were bled, and tongue and routine meat inspected; and serum antibody and parasite antigen concentrations were determined by ELISA. A model, based on a multinomial distribution and including all possible interactions between the individual tests required 31 parameters to be estimated, but actually allowed only 15 parameters (i.e. had 15 degrees of freedom) to be estimated. Therefore, prior expert opinion on specificity and (in)-dependence of the tests was entered in the model, resulting in a reduction of the number of parameters to be estimated. The estimated prevalence of porcine cysticercosis was 0.642 (95% confidence interval 0.54–0.91). The performances of the tests were (sensitivity (se)–specificity (sp)): tongue inspection (se 0.210–sp 1.000), meat inspection (se 0.221–sp 1.000), Ab-ELISA (se 0.358–sp 0.917), Ag-ELISA (se 0.867–sp 0.947). To validate the estimates obtained from the model we performed a second study: 65 randomly purchased Zambian village pigs were bled for serum antibody and antigen determination, their tongue and meat inspected; and in addition, the carcasses were dissected for total cysticercus counts (gold standard). Cysticerci were found in 31 pigs (prevalence 0.477, 95% confidence interval 0.35–0.60), overlapping with the estimated prevalence in the first study. Sensitivity and specificity values obtained for the aforementioned tests in this study were in agreement with those estimated. A Bayesian analysis framework offers the possibility to combine prior opinion with experimental data to more accurately estimate the real prevalence of porcine cysticercosis in the absence of a gold standard.

Keywords: Pig; *Taenia solium*; Cysticercosis; Bayesian methodology; Sensitivity; Specificity; Prevalence

1. Introduction

Taenia solium cysticercosis is considered to be a serious public health problem in endemic regions. The cysticerci of *T. solium* may lodge in the human brain causing cerebral cysticercosis (neurocysticercosis) resulting in headache, epileptic seizures, blindness, mental disturbance and even death (White, 2000).

Surveys in pigs have contributed to an increased awareness of this zoonotic infection in many developing countries, including eastern and southern Africa (Phiri et al., 2003). The comparability of these reports, however, is limited by major differences in methodology. Most of the findings are based on surveys using either tongue palpation or post-mortem examination. These techniques are reported to be specific but not very sensitive when applied on pigs with low cyst burdens (Gonzalez et al., 1990; Sciutto et al., 1998; Boa et al., 2002). In some surveys antibody or antigen

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detection by ELISA was used (e.g. Boa et al., 1995; Afonso et al., 2001; Ngowi et al., 2001; Phiri et al., 2002). These parasitological and serological tests have different sensitivities and specificities, making it difficult to compare these results. In addition, in most studies decision on infection was based on a single test. Unless a complete dissection of a pig carcass is performed, a gold standard for porcine cysticercosis is not available; therefore the true disease status cannot be accurately estimated using conventional methods.

Several authors have attempted to get a better estimate of the prevalence of a disease when the true disease status of this disease is unknown by combining the results of several tests in a model, including maximum likelihood estimation and Bayesian inference (Boelaert et al., 1999; Enøe et al., 2000). A major problem is that the use of imperfect diagnostic tests whose sensitivity and specificity are unknown in a particular situation entails the estimation of a number of parameters in excess of the number of degrees of freedom of the dataset. However, the number of parameters can be reduced through the application of constraints, based on expert opinion. A Bayesian analysis framework offers the possibility to combine prior opinion (expert opinion) with experimental data and estimate values for both prevalence and diagnostic test characteristics. It must be noted that the prior opinion is used to reduce the number of parameters to be estimated (thereby allowing estimation of prevalence and some of the less known test characteristics). This means that the prior information should be general enough to be applicable to the particular situation and precise enough to allow a reduction of the number of parameters to be estimated. In practice, this usually means, prior information on test specificity, and ideally the use of tests with specificity equal to one (the conditional probability of obtaining a negative result for a disease-free animal in any other test, given a positive result in the test in question, becomes irrelevant).

In this study a model was developed using a dataset of 868 Zambian village pigs in which infection with *T. solium* cysticercosis was measured by four different methods; tongue inspection, visual inspection of the carcass, serum antibody detection and serum antigen detection. Subsequently, the validity of this model was assessed externally with a dataset of another 65 pigs, that were purchased at local markets and in villages in Zambia, on which the same diagnostic methods were applied followed by a complete dissection of half the carcasses and all internal organs to count cysticerci and assess their viability.

2. Materials and methods

2.1. Animals

Study 1: Between March and June 2000, 868 pigs were examined and blood sampled at the Chibolya slaughter slab in Lusaka, Zambia. At this slaughter slab, only animals from resource-poor farmers in the rural areas, mainly the Southern Province of Zambia, are slaughtered. All pigs presented for slaughter on the days of sampling were included in the study.

Study 2: Between April and August 2001, 65 pigs were randomly purchased from the Chibolya slaughter slab in Lusaka ($n = 30$) and from villages in the Katete and Petauke districts (Sinda area) in the Eastern Province ($n = 35$).

The estimated age of the pigs in both studies varied between 6 months and several years; pigs of both sexes were sampled. Most pigs from Chibolya were crosses of large white and landrace breeds; those from the Eastern Province were all of local breed.

2.2. Tongue and carcass inspection

Tongue inspection was performed before slaughter: while the mouth was opened by using a wooden rod, the examiner, using a cloth, gently pulled the tongue, examined and palpated it throughout the base. The pig was considered positive for cysticercosis if cyst-like nodules were either seen or felt (Gonzalez et al., 1990).

Immediately following slaughter the presence of cysticerci in the carcass was assessed by examining *T. solium* cysticerci predilection sites, including the masseter muscles, triceps brachii muscle, tongue and heart, and *Taenia hydatigena* predilection sites, including the liver and peritoneum.

2.3. Slicing of tissues from carcasses and cysts

The muscle groups excised from the half carcasses together with the complete heart, tongue, head and neck muscles, psoas muscles, diaphragm, lungs, kidneys, liver, brains and eyes were sliced in such a way that all fully developed cysts could be revealed and enumerated (i.e. each slice was less than 0.5 cm thick). The total number of cysticerci for those muscle groups where cysts were only counted in half of the carcass was calculated by multiplying the detected unilateral number by two. Cysts that were encountered on incisional and intact surfaces were classified and enumerated as either viable (translucent, fluid-filled with invaginated whitish protoscolices visible) or degenerated (caseous or calcified) (Boa et al., 2002).

2.4. Serological tests

Blood samples were taken at slaughter from the jugular vein or the cranial vena cava and collected in plain vacutainer tubes; serum was separated by centrifugation and dispensed into aliquots and stored at $-20\,^{\circ}C$ until analysis.

2.4.1. Enzyme-linked immunosorbent assay for the detection of specific antibodies (Ab-ELISA)

The Ab-ELISA was performed using crude metacestode antigen of *Taenia crassiceps*. *T. crassiceps* cysticerci (Toi strain, kindly provided by Chernin (1975), and maintained in laboratory mice by two to four passages a year) were harvested from the peritoneal cavity of mice (Swiss A strain) approximately 90 days after experimental infection. The antigen was prepared according to Nunes et al. (2000). The ELISA was performed according to Nunes et al. (2000) with some minor modifications: all steps except the incubation of the substrate were done at 37 °C on a shaking plate, 30 min incubation for the coating of the antigen, 15 min for the other incubation steps; blocking was done using phosphate-buffered saline (PBS, pH 7.2) containing 0.05% Tween 20 and 2% newborn calf serum; test serum was diluted 1/200 in blocking fluid; anti-pig IgG (whole molecule) peroxidase conjugate (Sigma A-9417) was used at a dilution of 1/20,000 in blocking fluid; optical density was read at 492/655 nm. All samples were run in duplicate. On each plate two cysticercosis positive pig serum samples and eight negative pig serum samples were run. The cut-off was determined as the mean OD of the eight negative controls + 3 standard deviations.

2.4.2. Enzyme-linked immunosorbent assay for the detection of circulating antigen (Ag-ELISA)

The Ag-ELISA was performed as described by Dorny et al. (2000) with a few modifications: incubation steps were reduced from 1 h to 30 min (coating) or 15 min (other steps); all incubations were done on a shaking plate except for the last step (substrate); streptavidin–horseradish peroxidase (Jackson Immunoresearch lab Inc.) diluted 1/10,000 was used as the conjugate. The optical density of each serum sample was compared with a sample of negative pig serum samples ($n = 8$) at a probability due to chance (P) < 0.001 to determine the result of the test (Sokal and Rohlf, 1981).

2.5. Statistical analysis

Different models were constructed using the available expert opinion. These models will be reported elsewhere in detail (Berkvens et al., Estimating disease prevalence in a Bayesian framework using probabilistic constraints. In preparation). The models were run in WinBUGS 1.4 (Spiegelhalter et al., 2003). Model selection was based on the deviance information criterion and the number of parameters estimated in the model (respectively, DIC and pD) (Spiegelhalter et al., 2002).

A model, based on a multinomial distribution and including all possible interactions between the four individual tests requires 31 parameters to be estimated. These are the prevalence, the sensitivity and specificity of the first test, two conditional sensitivities and two conditional specificities for the second test, four conditional sensitivities and four conditional specificities for the third test, and finally eight conditional sensitivities and eight conditional specificities for the fourth test, i.e. $1 + 2 + 4 + 8 + 16 = 31$ (see Appendix A for normal description of parameters). This model is inestimable because the data (16 'classes' of test results) provide only 15 degrees of freedom. The model building strategy consists of incorporating the prior knowledge to reduce the number of parameters to be estimated. This is done by, in the first place, including priors that result in an explicit reduction of the number of parameters to be estimated: e.g. stating that the specificity of the test is equal to one automatically means that all parameters including the conditional term 1 can be dropped. Secondly, prior knowledge can be applied to reduce the possible range of values for a specific parameter. This reduction may furthermore affect the possible range of values for other parameters as well. Because of the complexity of the model, it is impossible to predict or calculate what level of reduction in the number of parameters to be estimated will result. DIC is used to evaluate the model fit. It is an information criterion and thus consists of two components: first an equivalent of a 'likelihood' measure (transformed in such a way that a lower value means a better fit) and second a penalty for the complexity of the model (the lower the value, the simpler the model). This information criterion is to be minimised during the model building process, attempting to find the best fit with the simplest possible model. The number of parameters that were effectively estimated represents the complexity of the model and this statistic is pD, which thus provides an indication of the final reduction in the number of parameters needed to be estimated.

In study 2 the sensitivities and specificities of the different tests were estimated on data from Southern and Eastern provinces. Exact confidence intervals were calculated using exact binomial distribution (StataCorp, 2001. Stata Statistical Software, Release 7.0. Stata Corporation 2001, College Station, TX). The effect of the origin of the pigs on test characteristics was measured by means of a logistic regression analysis (StataCorp, 2001. Stata Statistical Software, Release 7.0. Stata Corporation 2001, College Station, TX).

3. Results

3.1. Study 1

Of the 868 pigs examined and sampled at the Chibolya slaughter slab 115 pigs (13.2%) were positive by lingual inspection, 121 pigs (13.9%) were positive for *T. solium* cysticercosis on post-mortem examination, 216 (24.9%) had antibodies in the Ab-ELISA and 496 (57.1%) had circulating parasite antigen in the Ag-ELISA. In 400 pigs (46.1%) there was full agreement, either positive or negative, among all four tests. The infections diagnosed by carcass inspection were mostly massive with predominantly live cysts. Results

Table 1
Results obtained by four different tests for the detection of cysticercosis in village pigs slaughtered in the Chibolya slaughter slab, Lusaka, Zambia

No. of samples ($n = 868$)	Tests			
	Tongue inspection	Carcass inspection	Ab-ELISA	Ag-ELISA
326	−	−	−	−
281	−	−	−	+
42	−	−	+	−
95	−	−	+	+
0	−	+	−	−
0	−	+	+	−
5	−	+	−	+
4	−	+	+	+
1	+	−	−	−
2	+	−	−	+
0	+	−	+	−
0	+	−	+	+
2	+	+	−	−
35	+	+	−	+
1	+	+	+	−
74	+	+	+	+

(+) Indicates positive and (−) indicates negative results in test.

obtained by the four tests for the detection of cysticercosis are given in Table 1.

Using prior knowledge on specificity (specificity of tongue palpation and carcass inspection both equal to one; specificity of both Ab-ELISA and Ag-ELISA in excess of 0.90 (Nguekam et al., 2003b)) and dependence of the tests (tongue palpation and carcass inspection positively correlated in the infected pig population), the number of parameters to be estimated could be reduced to nine and the resulting model had minimal DIC (Spiegelhalter et al., 2002). Including information about the conditional dependence (i.e. higher probability of a positive result at carcass inspection when the animal is infected and the tongue inspection is positive) is an example of a constraint that is not perfect. In this model, this meant putting the range of possible values for parameter [4] at (0.8…1) and for parameter [5] at (0…0.2) (Appendix A). The effect of applying this expert opinion on the number of parameters to be estimated could be evaluated through pD.

The prevalence estimated by the model was 0.642 (95% confidence interval) for estimated prevalence 0.54–0.91). The performances of the tests were: tongue inspection, sensitivity (se) 0.210 (CI 0.14–0.26), specificity (sp) 1.000; meat inspection, se 0.221 (CI 0.15–0.27), sp 1.000; Ab-ELISA, se 0.358 (CI 0.26–0.41), sp 0.917 (CI 0.85–0.99); Ag-ELISA, se 0.867 (CI 0.62–0.98), sp 0.947 (CI 0.90–0.997).

3.2. Study 2

Study 2 was done to externally validate the estimates from the model obtained in study 1. Dissection of the half carcass was used as a gold standard for diagnosis of cysticercosis. Cysticerci were found in 31 of the 65 slaughtered pigs (prevalence 0.477; CI 0.35–0.60%), the range was 1–24,662 (Table 2).

In 10 pigs only viable cysticerci were found, in 14 viable and degenerated cysticerci and in seven only degenerated cysticerci. In 14 pigs less than 200 cysticerci (viable and degenerated) were counted; none of these cases were detected by routine meat inspection. *T. hydatigena* cysts were found in two pigs that were not infected with *T. solium* and in two pigs that were heavily infected with viable cysticerci of *T. solium*. These four cases were single

Table 2
Results of dissections of 65 Zambian village pigs (numbers, number of pigs in each category)

Test	Result	No cysticerci	*Taenia solium* viable cysticerci		*T. solium* only degenerated cysticerci	*Taenia hydatigena* cysticerci
			Light infection (1–100)	Moderate to heavy infection (>100)		
Tongue inspection	Pos	0	0	5	0	0
	Neg	32	10	9	7	2
Carcass inspection	Pos	0	0	12	0	0
	Neg	32	10	2	7	2
Ab-ELISA	Pos	4	0	9	4	0
	Neg	28	10	5	3	2
Ag-ELISA	Pos	1	7	14	0	2
	Neg	31	3[a]	0	7	0
Total (slicing of carcass)		32	10	14[b]	7	2

[a] Including one pig with 16 viable cysts, all in the brain.
[b] Including two pigs in which also *T. hydatigena* cysticerci were found.

Table 3
Agreement between the results obtained by four different tests for the detection of cysticercosis in 65 Zambian village pigs from which the infection was subsequently confirmed by dissection of the carcasses

No. of samples ($n = 65$)	Tests				Results of dissection				
	Tongue inspection	Carcass inspection	Ab-ELISA	Ag-ELISA	No cysts	Only viable *T. solium* cysticerci	Viable and calcified *T. solium* cysticerci	Only calcified *T. solium* cysticerci	*T. hydatigena* cysticerci
33	−	−	−	−	27	0	3	3	0
10	−	−	−	+	1	1	6	0	2
9	−	−	+	−	4	0	1	4	0
1	−	−	+	+	0	1	0	0	1
0	−	+	−	−	0	0	0	0	0
0	−	+	+	−	0	0	0	0	0
4	−	+	−	+	0	4	0	0	0
3	−	+	+	+	0	2	1	0	0
0	+	−	−	−	0	0	0	0	0
0	+	−	−	+	0	0	0	0	0
0	+	−	+	−	0	0	0	0	0
0	+	−	+	+	0	0	0	0	0
0	+	+	−	−	0	0	0	0	0
0	+	+	−	+	0	0	0	0	0
0	+	+	+	−	0	0	0	0	0
5	+	+	+	+	0	3	2	0	1

cyst infections of *T. hydatigena* with peritoneal localisation.

Agreement between the results obtained by the four tests for the detection of cysticercosis and the results of the dissection of the carcasses are given in Table 3.

There was total agreement among the four tests, either positive or negative, in 38 (58.8%) pigs. All four tests were negative in 33 pigs. In 27 (81.8%) of these no cysticerci were found; in the six others low numbers of mainly calcified cysticerci were detected. Five pigs had positive results in the four tests; these all had a relatively large burden of mainly viable cysticerci. In 10 animals, only the Ag-ELISA tested positive: among these, seven were infected with *T. solium*, two with *T. hydatigena* cysticerci, while one was not infected. In nine pigs, only the Ab-ELISA was positive: among these, one had viable and calcified cysticerci, four only calcified cysticerci, and four were not infected.

Sensitivity and specificity values obtained in this study were in agreement with those estimated: tongue inspection, (se) 0.161 confidence interval (CI 0.05–0.34), (sp) 1.000 (one-sided 97.5% confidence interval 0.90–1.00); meat inspection, (se) 0.387 (CI 0.22–0.58), (sp) 1.000 (one-sided 97.5% confidence interval 0.90–1.00); Ab-ELISA, (se) 0.452 (CI 0.27–0.64), (sp) 0.882 (CI 0.73–0.97); Ag-ELISA, (se) 0.645 (CI 0.45–0.81), (sp) 0.912 (CI 0.76–0.98). Logistic regression showed no differences in the prevalence and test characteristics between pigs from Southern and Eastern provinces ($P > 0.05$).

4. Discussion

In this study a Bayesian approach was used to estimate the prevalence of porcine cysticercosis on a dataset of 868 village pigs on which four diagnostic tests were performed. These pigs, sampled at a slaughter slab in Lusaka, were reared on resource-poor farms, mainly from the Southern Province of Zambia. A very high prevalence was estimated (64.2%, CI 0.54–0.91), much higher than previously reported in Zambia (20.6%) and other countries in the southern and eastern African region (between 5.1 and 45.0%). However, those earlier estimates were based on the results of a single test (Phiri et al., 2003).

The accuracy of this estimate was demonstrated through a second study that included a gold standard (dissection). The data for this study were collected from pigs purchased on the same slaughter slab in Lusaka and from local markets and resource-poor farms in the Eastern Province. No differences in the prevalence of cysticercosis and the test characteristics could be demonstrated between pigs from the Southern and Eastern Provinces. They are therefore considered to belong to the same population. The prevalence estimated from the model developed on the first dataset was in agreement with that observed in the second dataset, demonstrating the validity of the model and the possibility of estimating the prevalence of cysticercosis in the absence of a gold standard by combining the results of four tests. Dissection of a pig carcass followed by enumeration of cysticerci, considered here as the gold standard, is time-consuming and

expensive, and is therefore impractical for routine prevalence estimation.

The use of available prior information on specificity and dependence of the tests in the model reduced the number of parameters to be estimated to nine and yielded meaningful estimations on test characteristics. The fact that these estimates were confirmed in the validation study (study 2) is satisfying, but it must be remembered that posterior estimates in a Bayesian analysis are the result of both the data at hand and prior opinion, i.e. the prior expert opinion does influence the final estimates. It should also be obvious that the current approach does not in the first place attempt to update prior opinion on test characteristics, but uses this information to reduce the parameter space, allowing estimation of all remaining parameters (Berkvens et al., Estimating disease prevalence in a Bayesian framework using probabilistic constraints, In preparation). The validity of the prior opinion used in the present model, i.e. specificity of tongue palpation and carcass inspection both equal to one, specificity of the Ab-ELISA and Ag-ELISA greater than 90%, and tongue palpation and carcass inspection positively correlated in the infected pig population, was confirmed in the second study.

None of the light infections and only about half of the heavy and moderate infections could be detected by tongue inspection (Table 2). Routine carcass inspection was slightly better than tongue inspection in detecting moderate to heavy infections, but was equally insensitive in light infections. The existence of light infections in Zambian village pigs, together with the low sensitivity of the currently used parasitological techniques may result in a serious underestimation of the true prevalence of the infection and may explain the discrepancy between the reported prevalence in the region and the estimated prevalence in this study.

Within the past decade, highly reliable serological tests for the detection of human cysticercosis have been developed (Tsang et al., 1989; Ito et al., 1998, 1999), and these were also applied for immunodiagnosis of pigs (Tsang et al., 1991; Sato et al., 2003). Nunes et al. (2000) estimated the sensitivity and specificity values of ELISA, employing crude antigen from *T. cracciceps* metacestodes, at both 0.96. In the present model the sensitivity and specificity values of the Ab-ELISA were estimated at only 0.358 and 0.917, respectively. In Table 2 the inaccuracy of this test in detecting light infections is demonstrated: none of the light infected pigs had detectable circulating antibodies. The poor performance of this test, particularly with regard to sensitivity, when applied on village pigs with various intensities of infection, confirmed the results of Sciutto et al. (1998) in village pigs in Mexico. Semi-purified and purified antigens may show a higher specificity when applied in ELISA or the enzyme-linked immunoelectrotransfer blot technique (Tsang et al., 1991; Sato et al., 2003). However, it is doubtful whether these would increase the sensitivity when applied on these samples.

The performance of the Ag-ELISA was significantly higher than that of the other tests. The model resulted in estimates of 0.867 and 0.947 for the sensitivity and the specificity, respectively. Nguekam et al. (2003b) demonstrated that in experimentally infected pigs, circulating antigens were first detected between 2 and 6 weeks p.i., and remained present generally throughout an observation period of 6 months, even in pigs carrying only five to eight living cysts. In that study, the minimum number of living cysts, that could be detected using the Ag-ELISA, was one. It was noted that the sensitivity of the Ag-ELISA was lower (0.645) in study 2 than estimated in study 1 (0.867). This lower sensitivity can be explained by the fact that the statistical analysis did not differentiate between viable and degenerated cysts, and seven of the 31 pigs that were positive for cysticercosis had only degenerated cysts. In contrast to the Ab-ELISA, that measures exposure to the parasite, the Ag-ELISA detects only viable cysticerci (Nguekam et al., 2003b). This may be an advantage as the test may be used for monitoring the success of a therapeutic intervention in man and pigs (Benitez et al., 2001; Nguekam et al., 2003a).

Although the Ag-ELISA displayed a high specificity, both in the model and in the validation study, cross reactivity was observed with the metacestode stage of *T. hydatigena*. This is not surprising as the monoclonal antibodies used in the sandwich ELISA for the diagnosis of porcine cysticercosis were prepared against excretory-secretory antigens of the metacestode stage of the bovine tapeworm *Taenia saginata* (Brandt et al., 1992) and do consequently show genus—but not species—specificity. The large cysticerci of *T. hydatigena* are very common in small ruminants but rather uncommon in pigs in Africa. They are, however, much more widespread in pigs in Vietnam, thereby seriously impairing the usefulness of the Ag-ELISA in this country (Dorny et al., 2001).

In conclusion, this study has shown how a Bayesian approach can be used for obtaining better estimates of the prevalence of animal diseases and test characteristics by combining the results of imperfect diagnostic tests and expert opinion.

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Appendix A

Conditional probabilities

Prevalence	$\Pr(D^+)$	[1]
Se_1	$\Pr(T_1^+ \mid D^+)$	[2]
Sp_1	$\Pr(T_1^- \mid D^-)$	[3]
	$\Pr(T_2^+ \mid D^+ \cap T_1^+)$	[4]
	$\Pr(T_2^+ \mid D^+ \cap T_1^-)$	[5]
	$\Pr(T_2^- \mid D^- \cap T_1^-)$	[6]
	$\Pr(T_2^- \mid D^- \cap T_1^+)$	[7]
	$\Pr(T_3^+ \mid D^+ \cap T_1^+ \cap T_2^+)$	[8]
	$\Pr(T_3^+ \mid D^+ \cap T_1^+ \cap T_2^-)$	[9]
	$\Pr(T_3^+ \mid D^+ \cap T_1^- \cap T_2^+)$	[10]
	$\Pr(T_3^+ \mid D^+ \cap T_1^- \cap T_2^-)$	[11]
	$\Pr(T_3^- \mid D^- \cap T_1^- \cap T_2^-)$	[12]
	$\Pr(T_3^- \mid D^- \cap T_1^- \cap T_2^+)$	[13]
	$\Pr(T_3^- \mid D^- \cap T_1^+ \cap T_2^-)$	[14]
	$\Pr(T_3^- \mid D^- \cap T_1^+ \cap T_2^+)$	[15]
	$\Pr(T_4^+ \mid D^+ \cap T_1^+ \cap T_2^+ \cap T_3^+)$	[16]
	$\Pr(T_4^+ \mid D^+ \cap T_1^+ \cap T_2^+ \cap T_3^-)$	[17]
	$\Pr(T_4^+ \mid D^+ \cap T_1^+ \cap T_2^- \cap T_3^+)$	[18]
	$\Pr(T_4^+ \mid D^+ \cap T_1^+ \cap T_2^- \cap T_3^-)$	[19]
	$\Pr(T_4^+ \mid D^+ \cap T_1^- \cap T_2^+ \cap T_3^+)$	[20]
	$\Pr(T_4^+ \mid D^+ \cap T_1^- \cap T_2^+ \cap T_3^-)$	[21]
	$\Pr(T_4^+ \mid D^+ \cap T_1^- \cap T_2^- \cap T_3^+)$	[22]
	$\Pr(T_4^+ \mid D^+ \cap T_1^- \cap T_2^- \cap T_3^-)$	[23]
	$\Pr(T_4^- \mid D^- \cap T_1^- \cap T_2^- \cap T_3^-)$	[24]
	$\Pr(T_4^- \mid D^- \cap T_1^- \cap T_2^- \cap T_3^+)$	[25]
	$\Pr(T_4^- \mid D^- \cap T_1^- \cap T_2^+ \cap T_3^-)$	[26]
	$\Pr(T_4^- \mid D^- \cap T_1^- \cap T_2^+ \cap T_3^+)$	[27]
	$\Pr(T_4^- \mid D^- \cap T_1^+ \cap T_2^- \cap T_3^-)$	[28]
	$\Pr(T_4^- \mid D^- \cap T_1^+ \cap T_2^- \cap T_3^+)$	[29]
	$\Pr(T_4^- \mid D^- \cap T_1^+ \cap T_2^+ \cap T_3^-)$	[30]
	$\Pr(T_4^- \mid D^- \cap T_1^+ \cap T_2^+ \cap T_3^+)$	[31]

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