FULL PAPER

Parasitology

Epidemiological survey of *Toxoplasma gondii* and *Neospora caninum* infections in dairy goats in Central-Southern Taiwan

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ABSTRACT. *Toxoplasma gondii* and *Neospora caninum* are intracellular protozoan parasites that cause reproductive disorders in ruminants and humans. Information on the risk factors of *T. gondii* and *N. caninum* infections in goats is very limited in Taiwan. The aim of the study was to investigate the epidemiology and identify the risk factors of these two infections in goats. A total of 630 caprine sera were collected from 42 dairy goat farms and the owners were interviewed by a structured questionnaire. The apparent seroprevalences of *T. gondii* in farm- and individual- levels were respectively 88.1% and 32.22%, while those of *N. caninum* were 19.05% and 2.54%, respectively. *Toxoplasma gondii* B1 gene was identified in 7 feed samples and 8 from the water samples whereas *N. caninum* was not found. Wooden flooring was the main risk factor for *T. gondii* infection while the frequency of visits by staff to other farms and the breed of goat were risk factors for *N. caninum*. The improvement of flooring materials or thorough cleaning, periodic disinfection and maintenance of dryness on the floor are highly recommended for the prevention of *T. gondii* infection in farmed goats. In addition, unnecessary visits to other farms should be limited to prevent the spread of *N. caninum*. These factors should be highlighted for the prevention of *T. gondii* and *N. caninum* in goats, particularly when raised in intensive housing system with flooring on height.

KEY WORDS: goat, *Neospora caninum*, risk factor, seroprevalence, *Toxoplasma gondii*

Toxoplasmosis and neosporosis in farmed ruminants have been increasingly reported in recent years. Goats and sheep are highly susceptible to *Toxoplasma gondii* infection. Ingestion of oocyst or a tissue cyst containing bradyzoites has been shown to be the most common route for *T. gondii* transmission [7, 32]. Endogenous infection is recognized as the most common infective mode for *Neospora caninum*, and this is possibly triggered by down-regulation of cell-mediated immunity occurring around mid-gestation [19]. *Neospora caninum* infections may also occur via exogenous transplacental transmission through ingestion of oocysts shed by canids [18].

Three significant risk factors of toxoplasmosis were shown previously in ovine: 1) raising under intensive or semi-intensive conditions that increase the chance of rodents approaching to the feed; 2) feeding concentrate; 3) providing water from the public supply [35]. The presence and number of cats in the farms were indicated as important risk factors in another study [13]. It should also be noted that only few Norwegian goats in herds were antibody-positive at common level of exposure to *T. gondii*, an observation believed to be correlated with that Norwegian goats are vulnerable to infection during the gestation period [30].

Comparatively, population size was not recognized as a risk factor for toxoplasmosis in cattle farms in Southern Brazil [13], implicating that differences may exist between the risk factors of bovine and goat toxoplasmosis. On the other hand, it is well-known that *N. caninum* can cause serious illness in cattle but the economic and epidemiologic importance of *N. caninum* infection in goats remains uncertain as the pathogenesis of caprine neosporosis is largely unknown [27]. Epidemiological investigation revealed that herd- and individual-level seroprevalence in goats reached 75.2% and 10.7% respectively in Brazil [4], and graze at communal pasture, not using disposable syringes and flock size >25 goats were shown to be the risk factors for *N. caninum* infection in sheep and goats [28]. The presence of dogs was found to be a risk factor for small ruminant flocks [1]. In contrast, the presence of a calving pen, cold temperature climate, veterinary supervision and buying healthy animals to replace those culled can reduce the infection of *N. caninum* in small ruminants and cattle [1, 31].

In Taiwan, the first *T. gondii* infection in goats was confirmed by indirect fluorescence antibody test (IFAT) in 2000 [26] and a seroprevalence of 28.6% (30/105) was shown in 2011 [34]. Likewise, first data showing low *N. caninum* infection rate (1/25) by IFA in goats was reported in 2000 at central Taiwan [26]. Molecular prevalence of 22.3% (130/582) on *N. caninum* was documented in goat milk by conventional PCR and loop-mediated isothermal amplification PCR (LAMP) in 2013 [9]. As one of the mostly widespread zoonoses in the world, the risk of human toxoplasmosis through undercooked meat consumption never faded. Both toxoplasmosis and neosporosis should be cautiously concerned since most goat farms in Taiwan belong to intensive housing systems and loose pet dogs and cats on goat farms are highly common. Meanwhile, abortions with unknown causes still occur in caprine farms. To date, related studies on the risk factors of *T. gondii* and *N. caninum* in goats in either Asia or Taiwan are very limited. Analysis of risk factors associated with *T. gondii* and *N. caninum* in goat husbandry is crucial to minimize economic loss and to avoid the spread of associated diseases in animals and the zoonotic transmission of *T. gondii*. Hence, the aims of this study are to detect *T. gondii* and *N. caninum* infections in goats on serological level, and to further evaluate the risk factors of *T. gondii* and *N. caninum* infections in dairy goats. The data revealed in this study should strengthen further understanding of the epidemiology of *T. gondii* and *N. caninum* in goats and serve as the basis for strategies controlling these two infectious and hygienic diseases in Taiwan and neighboring countries.

MATERIALS AND METHODS

Animals and sample collection

The number of dairy goats in southwestern Taiwan, including Yunlin County, Chiayi County, and Tainan City accounted for 48% (25,099/52,343) of total dairy goats in Taiwan [38]. Goat farms from these three regions that belong to intensive housing systems with wooden or metal flooring high bed were recruited in this study to represent the dairy goat industry in Taiwan. Samples were collected by public veterinarians in regional animal disease control center between January and December, 2014. Forty-two out of 102 goat farms (Yunlin County: 6/24, Chiayi County: 14/29, Tainan City: 22/49) in sampling areas were recruited in this study. A total of 630 blood samples (15/each farm), 42 water samples, and 42 feed samples were collected from the farms. From each recruited farm, fifteen goat blood samples, 250–300 g of feed from feeding troughs and storage zone, and one liter of water from water troughs in stall were randomly collected and stored at 4°C for laboratory analyses.

Serological measurement of T. gondii and N. caninum antibodies

IgG immunoglobulin against *N. caninum* and *T. gondii* in sera were detected using commercial indirect ELISA kits (ID Screen® *Neospora caninum* Indirect Multi-species and ID Screen® Toxoplasmosis- Indirect Multi-species, IDvet, rue Louis Pasteur, Grabels, France). Sonicated lysate of *N. caninum* tachyzoites were prepared as antigen [3] and the *T. gondii* P30 antigen was coated on ELISA microplates for detection of specific antibody [8]. ID Screen® *Neospora caninum* comes with sensitivity 99.60% and specificity 97.30% in bovine. For ID Screen® Toxoplasmosis, the sensitivity and specificity was reported 82.48% and 97.89% in sheep, respectively [3, 24]. The ELISA was conducted according to the manufacturer's instructions. Sample to positive index (S/P) was calculated and interpreted as negative (S/P<0.40), positive (S/P≥0.50) or suspected (0.50>S/P≥0.40) for these two ELISA tests. Seroprevalence of *T. gondii* and *N. caninum* infections in dairy goats were determined at the individual and farm levels. A farm was recognized as positive when at least one seropositive animal was detected.

Isolation of T. gondii and N. caninum in feed and water samples

To detect oocysts attached on feed, the procedures for the detection of *Cyclospora cayetanensis* in vegetables developed by Food and Drug Administration [14] were conducted with modifications. Around 250 g feed on a stainless steel sieve (64 mesh/cm^2) were flushed with DDW to collect the oocysts from feed until 1 *l* filtered liquid was obtained. The filtered liquid was then treated with identical procedures as those for water samples described below [14, 21].

Water samples were placed at room temperature for 24 hr. A total of 200 m*l* of water with precipitates were added to four 50 m*l* tubes and centrifuged at 2,000 g at 4°C for 10 min. The supernatants were discarded and 1 m*l* pellets were combined, recentrifuged and mixed with equal volumes of lysis buffer for 1 hr with vortexing every 15 min. The sample was washed with DDW and then centrifuged twice at 2,000 g for 10 min at 4°C. The final pellet (1 m*l*) was stored at 4°C for DNA extraction [14, 21].

PCR identification of T. gondii and N. caninum isolated from feed and water samples

DNA was extracted from samples using Multisource Genomic DNA Miniprep Kit (Axygen Biosciences, Union City, CA, USA) according to the manufacturer's instructions with modification of incubation time (56°C, 1 hr for feed and water samples) and

proteinase K (final concentration of 0.6 mg/m*l*) [14, 21]. Genomic DNA extracted from the standard strains of *T. gondii* (ATCC® 50174 *Toxoplasma gondii* RH) and *N. caninum* (ATCC® 50843 *Neospora caninum* Nc-1) were used as respective positive controls. The primers for DNA amplification of *T. gondii* and *N. caninum* are applied in current study according to previous references [5, 6, 12, 25]. The reaction mixture with DNA extract of samples or standard strains, primers Np6+ and Np21+, *Taq* DNA polymerase, 10X *Taq* Buffer, deoxynucleotide triphosphates (dNTP), dimethyl sulfoxide (DMSO) and DDW were prepared to carry out the PCR for detection of *N. caninum* as described previously [25]. A nested PCR (nPCR) for the detection of the *ITS1 gene* of *N. caninum* was also accomplished [12]. Meanwhile, the nPCRs were performed to detect the *B1* gene and of *ITS1* gene of *T. gondii* in the feed and water samples as previously shown [5, 6]. After PCR amplification, 5 *μl* of each PCR product was electrophoresed by 2% agarose gel at 100 V for 30 min to identify the size of products.

Verification of T. gondii B1 Gene

The sequences of PCR positive products of *B1* gene, together with the standard strains (positive control), were verified by Sequencing Core Laboratory in Medical College, National Cheng Kung University. The acquired sequences were compared with the databases on National Center for Biotechnology Information (NCBI) website, using BLAST® program (http://blast.ncbi.nlm.nih.gov/).

Questionnaire

An organized questionnaire was designed to collect general (farm owner's name, farm address, telephone number), geo-environmental (geography, water supply, surroundings), management (frequency of disinfection, degree of cleanliness), animal (population size, presence of cats and dogs, rodent appearance) and worker (number of staff, veterinary service) information referred to the questionnaire design in previous studies [13, 17, 28, 31]. All of the questionnaires were completed by the same interviewer through personal interview.

Statistical analyses

For descriptive analyses, farm- and individual-level seroprevalence were calculated. Univariate logistic regression analyses were firstly used to examine the association between *T. gondii*/*N. caninum* infection and 16 variables, which were listed in Table 1. These 16 variables include location, near roads/other farms, feed storage, delivery room, handling methods of abortive tissue, rodent control, frequency of visits by staff to other farms, frequency of veterinarian service, resident, breed, flooring, dog approaching to feed, cat approaching to feed, dog/cat approaching to feed, vehicles entering the farm, and disinfection of entering vehicles. To determine risk factors of *T. gondii*/*N. caninum* infection in multivariate level, the variables with significant results of univariate models were included in a multivariate regression model. *P*-values less than 0.05 were considered statistically significant. Statistical analyses were performed by utilizing SPSS version 20.0 (SPSS Inc.; Chicago, IL, USA).

RESULTS

Seroprevalence of T. gondii and N. caninum

Seroprevalence of *T. gondii* infection was higher than that of *N. caninum* infection at both farm- and individual-level in most farms (Table 2). The apparent seroprevalence of *T. gondii* in individual-level was 32.22% (203/630), indicating the true seroprevalence as 34% (95% CI: 30.36–37.64). The apparent seroprevalence of *N. caninum* in individual-level was 2.54% (16/630), suggesting the true seroprevalence as 0%. The seroprevalences of *T. gondii* and *N. caninum* antibodies in farm-level were 88.1% and 19.05%, respectively. Seroprevalence of *T. gondii* and *N. caninum* infections represented discrepancy among three investigated regions. The highest *T. gondii* infection and lowest *N. caninum* infection was found in Yunlin County, while the prevalent status of both parasites was similar in Chiayi County and Tainan City.

Molecular evidence of T. gondii and N. caninum in feed and water

Among 60 feed samples (23 from feed trough and 37 from feed storage, respectively) and 41 water samples, the target sequences of *B1* gene of *T. gondii* were validated in 7 feed (4 from feed trough and 3 from feed storage) and 8 water samples. *N. caninum* was not found in both feed and water samples (Fig. 1 and Table 3). When blasted against the NCBI database, all sequences were shown to be of Toxoplasma *B1* gene origin with minor variation, which were 3 single nucleotide polymorphisms (SNPs) showed in 4 haplotypes with 55 bases in length. The haplotypes could not be classified according to the sample types (feed or water) or collection locations (Supplementary Fig. 1).

The risk factors of T. gondii and N. caninum infections

In univariate logistic regression analysis, flooring materials were significantly related to *T. gondii* infection, while two variables, frequency of staff visiting other farms and goat breed, were found to be significantly related to *N. caninum* infection (Table 1). The farms in which staff visiting other farms at least once per week were 5.8 times (OR=5.8, *P*=0.040) more likely to be *N. caninum* positive than the farms in which staff visiting other farms less than one time per week. Compared to mixed breed goat farms, pure breed ones were 6.43 times (OR=6.43, *P*=0.028) more likely to be *N. caninum* positive farms. The risk of *T. gondii* infection in farms with wood flooring were 6.44 times (OR=6.44, *P*=0.035) higher than farms with metal flooring. The results of multivariate logistic regression analysis suggested that goat breed (OR=14.41, *P*=0.022) and frequency of staff visiting other farms (OR=14.19, *P*=0.027) were both significant determinants of *N. caninum* infection (Table 4).

Table 1. Univariate analysis of variables related to *Toxoplasma gondii* / *Neospora caninum* infection in dairy goats

Variable	*Toxoplasma gondii*		*Neospora caninum*	
	Odds ratio	*P*-value	Odds ratio	*P*-value
Location				
Yunlin	Reference			
Chiayi	3.00	0.341	4.41	0.999
Tainan	3.17	0.279	4.75	0.999
Near roads/other farms				
No	Reference			
Yes	1.67	0.572	1.83	0.445
Feed storage				
Feed storage in warehouses or bins	Reference			
Feed storage inside the farm	1.78	0.492	0.79	0.764
Delivery room				
Yes	Reference			
No	1.30	0.770	0.23	0.193
Handling methods of abortive tissue				
Rendering	Reference			
Feeding to dog	3.90	0.999	1.38	0.793
Burning or burying	3.90	0.999	0.00	0.999
Rodent control				
Yes	Reference			
No	1.67	0.572	0.48	0.403
Frequency of visits by staff to other farms				
<1 time/week	Reference			
≥1 time/week	1.78	0.618	**5.8**	**0.040** [a]
Frequency of veterinarian service				
≥1 time/month	Reference			
<1 time/month	0.77	0.770	0.22	0.064
Resident				
Not near the farm	Reference			
Live near the farm	0.32	0.316	6.15	0.999
Breed				
Mixed	Reference			
Pure breed	1.00	1.000	**6.43**	**0.028** [a]
Flooring				
Metal	Reference			
Wooden	**6.44**	**0.035** [a]	0.93	0.930
Dog approaching to feed				
No	Reference			
Yes	0.20	0.152	0.62	0.544
Cat approaching to feed				
No	Reference			
Yes	1.26	0.783	1.13	0.881
Dog/Cat approaching to feed				
No	Reference			
Yes	0.42	0.444	0.51	0.424
Vehicles entering the farm				
No	Reference			
Yes	0.00	0.999	4.17	0.999
Disinfection of entering vehicles				
No	Reference			
Yes	0.93	0.940	0.95	0.950

a) *P*<0.05

Table 2. Seroprevalence of *Toxoplasma gondii* and *Neospora caninum* infections in dairy goats

Region	Pathogen	Farm level (%)	Individual level (%)	
		Prevalence	Apparent prevalence	True prevalence (95% CI)
Yunlin	*T. gondii*	100 (6/6)	53.33 (48/90)	57.91 (47.71–68.11)
	N. caninum	0 (0/6)	0 (0/90)	0
Chiayi	*T. gondii*	85.71 (12/14)	24.29 (51/210)	25.01 (17.26–32.76)
	N. caninum	21.43 (3/14)	1.9 (4/210)	0
Tainan	*T. gondii*	86.36 (19/22)	31.52 (104/330)	33.20 (28.80–37.60)
	N. caninum	22.73 (5/22)	3.64 (12/330)	0.97 (0.05–1.88)
Total	*T. gondii*	88.10 (37/42)	32.22 (203/630)	34.00 (30.36–37.64)
	N. caninum	19.05 (8/42)	2.54 (16/630)	0

Table 3. Positive rate of *Toxoplasma gondii* and *Neospora caninum* in dairy goat farm environment (feed and water)

Sample \ Gene	*T. gondii*		*N. caninum*	
	B1	ITS1	Nc5	ITS1
Feed trough (n=23)	17.39% (4/23)	0	0	0
Feed storage (n=37)	8.11% (3/37)	0	0	0
Water (n=41)	17.07% (7/41)	2.44% (1/41)	0	0

Table 4. Multivariate logistic regression for risk factors related to *Neospora caninum* infection in dairy goats

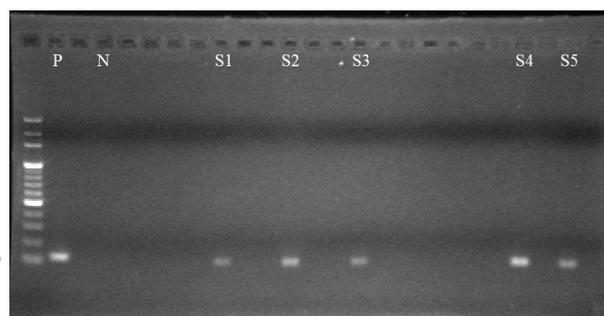| Risk factors	Odds ratio (95% CI)	*P*-value
Frequency of staffs visiting other farms		
<1 time/ week	Reference	
≥1 time/ week	14.19 (1.35–149.76)	0.027
Breed		
Mixed	Reference	
Pure breed	14.41 (1.47–141.35)	0.022

Fig. 1. Amplification of *B1* gene from water and feed samples. The target bands were observed from 5 collected samples at 94 bp (S1–5: positive samples; P: positive control; N: negative control).

DISCUSSION

Seroprevalence of heteroxenous parasites *T. gondii* and *N. caninum* has been examined in different areas worldwide due to their well-known relationships with abortions in goats [11, 22, 36]. In the current investigation, the average goat population size was 340, which is much larger than that in Norway (83 goats per herd) [31]. In Taiwan, goat barn with flooring was commonly used in the intensive raising system. Namely, dairy goats are intensively raised in the goat shed with metal or wooden flooring on height in our studied areas. We found that the wooden floor was significantly associated with higher seroprevalence (6.44-fold) of *T. gondii* infection, compared with the metal floor. The reasons could be that wooden floor produces more dead space that is favorable for the protozoan oocysts in feces, which makes thorough disinfection against the parasite relatively difficult [16]. A previous study showed that *T. gondii* oocysts survive at higher relative humidity (RH) in wood (8–25%, related with RH of air) for a long time but were killed in drying at RH of 19% within 11 days [20], which is coherent with the finding of risk factor on caprine *T. gondii* in the current investigation. Risk factor analysis of *T. gondii* infection displayed that metal flooring was significantly related to lower seroprevalence, showing that possibly less contamination and better cleaning and disinfection on the metal flooring, which is in agreement with the finding in sheep by Skjerve *et al.* in Norway [29]. Also, since dairy goats are intensively raised on the flooring in goat houses on height, the surfaces that the goats most frequently contact with are the metal or wooden flooring. It is harder to accomplish extensive cleaning and disinfection on the wooden flooring due to tiny natural interstices or meshes on the wood, thereby more easily containing *T. gondii* oocysts from contaminated soil, water or plant materials like hay. That may be the main reason that flooring materials are a risk factor for *T. gondii* infection in farmed goats in the current study. Therefore, to avoid possible spread of *T. gondii* via wooden flooring in the goat house, several strategies can be executed. Improvement of flooring

materials should be a feasible manner. Meanwhile, periodic disinfection and maintenance of dryness on the floor also should be implemented for the short- and mid-term prevention and control of *T. gondii* in the intensive goat raising system.

Most farms in the current study were not purely dairy goat farms as the owners also raised male goats for meat production. Alpine and Saanen were the main breeds in our study and both breeds are famous for milk production. The seroprevalence of *N. caninum* infection was associated with the frequency of contacts with employees and goat breeds of other farms. The risk factor of caprine breed for *N. caninum* infection is similar with the data by Santos *et al.* that either pure or mixed breeds were a risk factor [28] while another report also showed that Damascus was more sensitive to neosporosis than the mixed breed [2]. The current and previous data together demonstrated that the breed of goat is highly associated with *N. caninum* infection. Moreover, multiple logistic regression analysis of *N. caninum* infection demonstrated that the frequency of visits by staff to other farms and goat breeds were also risk factors. Dogs and related canids have been found as definitive hosts of *N. caninum.* Recent investigation in Colombia has shown that horizontal transmission is a crucial route for *N. caninum* infection in cattle [23]. Meanwhile, we have shown that stray dogs are a social and hygienic issue in Taiwan and the number of pet dogs have clearly increased during the years 2009 to 2014 [15]. Similarly, visiting live animal market is a significant factor for *N. caninum* in goats by the univariable analysis [2] and workers visiting other farms was a risk factor for *N. caninum* infections in cattle in Jordan [1, 31]. Thus, we assumed that high frequency of visits to other farms or live animal markets may increase the chances to carry contaminated soil or water derived from stray or pet dogs in other farms and neighboring areas. This consequently enhanced the possibility of *N. caninum* infections in farmed goats.

On the other hand, no significant differences in seroprevalence of *T. gondii* and *N caninum* infection were observed among the three investigated areas in Taiwan. Farm-level seroprevalence of *T. gondii* infection was higher than its individual-level (88.10% vs. 34.00%), indicating widespread of *T. gondii* infection in dairy goats. Moreover, high farm-level coupled with lower individual-level seroprevalence was observed in Chiayi County and Tainan City, similar to previous studies in Norway and Poland [10, 30]. This occurred possibly because, during the gestation period, goats are vulnerable to *T. gondii* infection by potential exposure to cat feces or other contaminated feed. Our study indicates that wooden flooring is a risk factor for *T. gondii* so raising goats on the wooden materials could possibly enhance chances of contact with pathogen in these farms. Although the studies on seroprevalence of toxoplasmosis in goats in China are limited, it should also be noticed that the individual seroprevalence of *T. gondii* was also high in Qinghai province of China (29.54%, 192/650) [22]. This implicated that the possibility of similar *T. gondii* prevalence in goats occurs in some areas of China and in Taiwan. In addition, antibodies against *N. caninum* infection were rarely seen at both farm- (prevalence: 19.05%) and individual-level (true prevalence: 0%), similar results were also shown by Santos *et al.* [28] (flock 16.4%; individual 2.7%) and Abo-Shehada and Abu-Halaweh [1] (flock 12%; individual 2%). These data together revealed a convincing epidemiological profile for *N. caninum* in goats. Reasonable explanations are that goats are less susceptible to *N. caninum*, compared to cattle. Consistently, DNA of *N. caninum* was not found in organ, feed and water samples in the current study.

Molecular biological methods for detection of *T. gondii* in water has been developed and evaluated in several studies [21, 37]. In this study, we first detected *T. gondii* in water and feed in Taiwan, and the results showed somewhat higher positive rate in the water (19.51%, 8/41) than that in the feed (11.67%, 7/60), indicating possible transmission routes of *T. gondii* through both water and feed. On the other hand, the method developed by Food and Drug Administration for detection of *Cyclospora cayetanensis*, a coccidian protozoon in vegetables, was employed with modifications in the current study [14]. Approximately 17% (4/23) *T. gondii* DNA was detected in feed trough samples, which is two times more than that found in feed storage (8.10%, 3/37). *N. caninum* DNA was not found in any feed samples. According to our risk factor analysis data and considering the similarity between two oocysts in the environment resistance, one of the reasons might be that using the wooden flooring as background on height in the intensive management system, the feed might be more possibly contaminated in the trough.

In the questionnaire investigation coupled with risk factor analysis, the relations between of *T. gondii* and *N. caninum* infections were also examined. Despite similar transmitting route, no correlations between these two infections were observed. This is reasonable since the two parasites have different final hosts and their respective final hosts possess quite different behavior on the farm. Moreover, susceptibility of goats to *T. gondii* is much higher than that to *N. caninum*. In this study, neither presence nor number of cats and dogs on farms were recognized as the risk factors of *T. gondii* and *N. caninum* infection, which is in disagree with other studies [22, 33]. The sensible reasons could be that in the previous investigations, goats were grazing in nearby pastures and thus likely to have direct contact with dogs and cats as well as their feces. Goats were therefore infected by consuming grass, feed and water contaminated by protozoal oocysts. In contrast, in our investigated areas, goats were intensively raised on flooring on height most of the time and thus have lower opportunity to contact with the dog and cat feces directly. Another reason was that even though no cat/dog was recorded in the questionnaire, unnoticed presence of stray cats/dogs on the farm is still possible; this suggestion is supported by the study of Tzanidakis *et al.* [35]. The crucial roles of cats and dogs in *T. gondii* and *N. caninum* transmission have been widely and commonly recognized so it is quite possible that their importance was masked by other factors like sources of feed and water contamination, and consequently failed to show statistical significance in the risk factor analysis.

The data of serological and molecular examinations in the current study demonstrated that *T. gondii* infection was much more severe than *N. caninum* in dairy goats in Central-southern Taiwan. Risk factor analysis indicated that the materials of flooring on height play a crucial role in the prevention and control of caprine *T. gondii* infection in the intensive raising system. Decrease in farm employee entry into other farms may be helpful to reduce *N. caninum* infections in dairy goats. Although in the current study the presence of cats and dogs on farms was not identified as a risk factor in Taiwan, the impact of pet or stray cats/dogs should not be overlooked since contaminations of feed and water were present. The work revealed epidemiological understanding of *T. gondii*

and *N. caninum* in the goat industry of intensive management and the current data could serve as a pivotal basis for the prevention of the two protozoal infections in goats raised on the similar systems in both veterinary and hygienic aspects.

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