Original Article

Survey of *Toxoplasma gondii* in Taipei: Livestock Meats, Internal Organs, Cat and Dog Sera

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Abstract

Toxoplasma gondii (*T. gondii*) is an important food-borne zoonotic protozoa. Infection occurs when humans and warm-blooded animals consume raw or lightly cooked cysts containing meat or sporulated oocysts contaminated food. *T. gondii* infection has been observed in food animals in many countries suggesting the significance of food animals in the epidemiology of toxoplasmosis. However, there is no report regarding *T. gondii* infection in food animals other than pigs in Taiwan. Therefore, in this study, pork, pig livers, mutton, chicken flesh, chicken hearts, chicken livers, gizzards, and imported beef sold in the supermarkets in Taipei were examined for T. gondii deoxyribonucleic acid (DNA) by polymerase chain reaction (PCR), for *T. gondii* antigens by enzyme-linked immunosorbent assay (ELISA) and for cysts by microscopy. Because *T. gondii* infected cats may shed oocysts in their feces and the mechanical spread of *T. gondii* to humans can also be conducted by dogs, serosurvey was also conducted on cats and dogs. The results showed that the prevalence of *T. gondii* DNA were 8% in pork, 2% in pig liver, 4% in mutton, 4% in chicken flesh, 2% in chicken heart, and 5% in imported beef. However, *T. gondii* antigens were not detected in all samples and cysts were not found in DNA positive samples. The seroprevalence of antibodies to *T. gondii* in cats and dogs were 10% and 6% respectively which are not significantly different from those reported in the year 1998. This is the first survey on *T. gondii* in livestock meats and internal organs in Taipei.

Keywords: cat, dog, livestock, serum, Taipei, *Toxoplasma gondii*

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บทคัดย่อ

การสำรวจเชื้อ *Toxoplasma gondii* ในเมืองไทเป จากตัวอย่างผลิตภัณฑ์เนื้อสัตว์ อวัยวะภายใน ซีรั่มของสุนัขและแมว

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Toxoplasma gondii (*T. gondii*) คือเชื้อโปรโตซัวที่สำคัญที่ติดต่อจากสัตว์สู่คนผ่านทางอาหาร การติดเชื้อเกิดขึ้นเมื่อคนและสัตว์เลือดอุ่นกินอาหารดิบหรือสุกๆดิบๆ ที่มีการปนเปื้อนของซิสต์หรือโอโอซิสต์สุก (sporulated oocyst) การติดเชื้อ *T. gondii* สามารถพบได้ในสัตว์ที่เลี้ยงเป็นอาหารในหลายประเทศซึ่งบอกถึงความสำคัญของสัตว์ที่เลี้ยงเป็นอาหารในด้านระบาดวิทยาของ toxoplasmosis อย่างไรก็ตาม ยังไม่มีรายงานการติดเชื้อ *T. gondii* ในสัตว์ที่เลี้ยงเป็นอาหารอื่นๆ นอกเหนือจากสุกรในไต้หวัน การศึกษาครั้งนี้ทำการตรวจหาสารพันธุกรรมของเชื้อ *T. gondii* โดยปฏิกิริยาลูกโซ่โพลิเมอร์ (polymerase chain reaction) ตรวจหาแอนติเจนของเชื้อ *T. gondii* โดยวิธี ELISA และตรวจหาซิสต์ด้วยกล้องจุลทรรศน์ จากเนื้อสุกร ตับหมู เนื้อแกะ เนื้อไก่ หัวใจไก่ ตับไก่ กระเพาะบด และเนื้อวัวนำเข้า ที่ขายในซูเปอร์มาร์เก็ตในไทเป จากการที่แมวที่ติดเชื้อ *T. gondii* สามารถขับโอโอซิสต์ได้ในอุจจาระ และการติดต่อของเชื้อยังเกิดจากสุนัขสู่คนได้ ดังนั้นจึงทำการสำรวจด้านซีรั่มวิทยาในแมวและสุนัขร่วมด้วย ผลการศึกษาความชุกของโรค พบสารพันธุกรรมของเชื้อ *T. gondii* ในเนื้อสุกรร้อยละ 8 ตับหมูร้อยละ 2 เนื้อแกะร้อยละ 4 เนื้อไก่ร้อยละ 4 หัวใจไก่ร้อยละ 2 และเนื้อวัวนำเข้าร้อยละ 5 อย่างไรก็ตามไม่พบแอนติเจนของเชื้อ *T. gondii* ในตัวอย่างที่ทำการตรวจ และไม่พบซีสต์ในตัวอย่างที่พบสารพันธุกรรม สำหรับผลการสำรวจด้านซีรั่มวิทยาโดยตรวจหาแอนติบอดี้ต่อเชื้อ *T. gondii* ในแมวและสุนัข พบว่าได้ผลเท่ากับร้อยละ 10 และ 6 ตามลำดับ ซึ่งไม่แตกต่างจากที่รายงานในปี 1998 การศึกษาครั้งนี้ถือเป็นการตรวจหา *T. gondii* ครั้งแรกในเนื้อสัตว์เศรษฐกิจและอวัยวะภายใน ในไทเป

คำสำคัญ: แมว สุนัข ปศุสัตว์ ซีรั่ม ไทเป *Toxoplasma gondii*

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Introduction

Toxoplasma gondii (*T. gondii*), an obligate intracellular protozoa, is an important food-borne zoonotic protozoa. Infection occurs when humans and warm-blooded animals consume raw or lightly cooked cysts containing meat or sporulated oocysts contaminated food. *T. gondii* may cause abortion in food animals, cerebral and ocular lesions in children with perinatal infection, and fatality in immunocompromised individuals (Davidson, 2000). *T. gondii* tissue cysts are rendered non-viable when meat is frozen (-12^{0}C), heated (internal temperature 66^{0}C), or irradiated (0.5 kilogray). Currently, no drugs are available to kill *T. gondii* cysts (Dubey, 1988; Dubey, 1996; Dubey et al., 2005).

Toxoplasmosis has been observed in food animals in many countries indicating the significance of food animals in the epidemiology of toxoplasmosis. *T. gondii* has been frequently observed in pork or pig organs (heart, brain, liver, spleen) (Pop et al., 1989; Choi et al., 1997; Dias et al., 2005). In the ruminants, *T. gondii* has been identified in muscle, heart, liver and lung of cattle (Pop et al., 1989; Dubey, 1992; Arias et al., 1994; Moré et al., 2008) and in muscle of sheep (Pop et al., 1989). Attention must also be paid to the potential importance of chicken flesh and internal organs in public health because *T. gondii* organisms have been found in chicken flesh, heart, liver and gizzard (Kaneto et al., 1997; Deyab and Hassanein, 2005; Dubey et al., 2007). It has been shown that *T. gondii* cysts are more often isolated from pork than beef and chicken (Dubey et al., 2005). Nevertheless, there is no significant difference in the bioassay of the

muscle of pig, cattle and sheep as to the presence of *T. gondii* in different animal species (Pop et al., 1989).

The widespread *T. gondii* infection in Taipei has been shown by serologic survey with antibody prevalence being 7% for humans (Lin et al., 1998), 7.9% for pet dogs (Lin, 1998), 8% for stray dogs (Lin et al., 2004), 7.7-14% for pet cats (Lin et al., 1990; Lin et al.,1998), and 37% for stray cats (Lin et al., 1998). In Taipei Zoo, as high as 38.75% of animals and 8.48% of employees were seropositive (Lin et al., 2009; Liao et al., 2011). Recent surveys in Taiwan also found that 10.1-28.8% of pigs (Fan et al., 2004; Tsai et al., 2007) and 4.7% of pigeons (Tsai et al., 2006) were *T. gondii* seropositive. However, there is no report regarding infection condition in food animals other than pigs.

In multivariate analysis, the most significantly increased risk of *T. gondii* infection is related to consuming undercooked meats and internal organs (Baril et al., 1999; Lee, 2000; Jones et al., 2009). So far, data concerning the prevalence of *T. gondii* in retail livestock meats and internal organs are not available in Taipei. Therefore, in this study, pork, pig livers, mutton, chicken flesh, chicken hearts, chicken livers, gizzards and imported beef which are frequently consumed by people in Taipei were examined for *T. gondii* deoxyribonucleic acid (DNA) by polymerase chain reaction (PCR) assay and for *T. gondii* antigens by enzyme-linked immunosorbent assay (ELISA).

In addition, commercial raw meat diets containing *T. gondii* cysts may cause food-borne infection in pets. The potential risk of human infection is closely related to the infected pets and their environments. (Strohmeyer et al., 2006). Infected cats may shed oocysts in their feces (Davidson, 2000). The mechanical spread of *T. gondii* to humans can also be conducted by dogs via ingesting or rolling in cat feces contaminated with oocysts (Lindsay et al., 1997). Therefore, the prevalence of *T. gondii* infection in pets should be viewed as a potential indicator when investigating the degree of the *T. gondii* contamination in the environments. Since the last survey on pet cats and dogs had been conducted in the year 1998 (Lin, 1998; Lin et al., 1998), therefore, serosurvey on *T. gondii* was also performed in pet cats and dogs. To improve the specificity of antibody detection, ELISA in combination with immunoblotting was used to detect antibody to a unique 30 kilodalton (kD) *T. gondii* surface antigen, P30 (Lin and Su, 1997).

Materials and Methods

Collection of samples: One hundred samples of pork, pig livers, mutton, chicken flesh, gizzards, chicken livers, chicken hearts and imported beef were purchased from three supermarkets in Taipei City. These meats and internal organs are usually frozen before being put on supermarket refrigerator shelf. Six pieces of one-gram sample were randomly cut from each livestock meat or internal organ. Two were subjected to nested PCR for DNA detection, two were examined by ELISA for antigen detection, and the rest were stored at -70^{0}C for microscopic examination for cysts when DNA or antigen detection was positive.

One hundred samples of venous blood were obtained from cats and dogs correspondingly at the National Taiwan University Animal Hospital. After clotting at 4^{0}C overnight, sera were collected for antibody detection. This study was performed in the period of 2008-2010.

Preparations of T. gondii DNA and antigens

Preparations of T. gondii DNA as positive control for nested PCR: The maintenance of the RH strain of *T. gondii* has been described previously (Lin, 1998). *T. gondii* tachyzoites were lysed in buffer containing 0.5% sodium dodecyl sulfate (SDS), 100 mM sodium chloride (NaCl), 10 mM ethylene diamine tetra-acetic acid (EDTA), 10mM Tris-Cl (pH 8.0), and 0.1 mg/ml proteinase K (Sigma Chemical Co, St. Louis, MO, USA). DNA was extracted with phenol-chloroform after incubation for 4 hours at 55^{0}C. DNA was then precipitated with 3M sodium acetate at -20^{0}C. After centrifugation for 10 min at the speed of 10,000 xg, DNA pellet was washed in 70% ethanol and mixed with TE buffer (1 mM EDTA, 10 mM Tris-Cl, pH 8.0). DNA was purified again and the resulting pellet was mixed with TE buffer. DNA concentration was measured by UV absorption (260/280 nm). The quantified *T. gondii* DNA would be used as positive control for nested PCR.

Preparations of T. gondii whole antigens for antibody-ELISA (kinetics-based ELISA) and as positive control for antigen-ELISA (avidin-biotin ELISA): In preparation of soluble whole *T. gondii* tachyzoite antigens, tachyzoites were suspended in phosphate-buffered saline (PBS), and treated with freezing-thawing (three cycles) and ultra-sonication (10 times, 35 watts/30 sec each) (Heat Systems Inc., Farmingdale, NY, USA). After centrifugation for 40 min at the speed of 10,000 xg at 4^{0}C, soluble whole tachyzoite antigens were collected. Following measurement of protein concentrations (Bio-Rad Lab, Richmond, CA, USA), whole tachyzoite antigen solution was stored at -70^{0}C until used.

Preparations of T. gondii membrane antigens for immunoblotting: In preparation of soluble membrane antigens for immunoblotting, tachyzoites were suspended in Nonidet-P40 (1% in 50 mM Tris, pH 8.0; Sigma Chemical Co.). After incubation for 12 hours at 4^{0}C, the suspensions were centrifuged for 40 min at the speed of 10,000 xg at 4^{0}C. Soluble membrane antigens were collected. Following measurement of protein concentrations (Bio-Rad Lab), membrane antigen solution was stored at -70^{0}C until used.

Examination of T. gondii in livestock meats and internal organs

T. gondii B1 gene detection by nested PCR: Two pieces of one-gram sample were used. Each one gram of sample was added to the last amount of 1 ml digestion solution containing proteinase K (0.1 mg/ml), SDS (0.5%), EDTA (10 mM), NaCl (100 mM), and Tris-Cl (10mM, pH 8.0) (Sigma Chemical Co.). Samples were then homogenized, and incubated at 55^{0}C for 18 hours with shaking. Samples were then mixed vigorously and incubated at 55^{0}C for 4 hours

with shaking. After centrifugation for 10 min at the speed of 10,000 xg, the supernatants were stored at -20^{0}C until used. Amplification of DNA was carried out in a 100-µl reaction mixture containing sample fluid and reaction buffer (50 pmol of each primer, 250 µM of each dATP, dGTP, dCTP, dTTP, 2.5 units of Taq DNA polymerase, 2 mM $MgCl_2$). All reagents were purchased from Promega Co, WI, USA. Four oligonucleotides used were: primer 1: 5'-GGAACTGCATCCGTTCATGAG-3', primer 2: 5'-TGCATAGGTTGCAGTCACTG-3', primer 3: 5'-GGCGACCAATCTGCGAATACACC-3' and primer 4: 5'-TCTTTAAAGCGTTCGTGGTC-3'. Primers originated from *T. gondii* B1 gene were used because this gene is conserved in all isolates found and is also present as a minimum 35-fold in the genome. Genomic DNA was amplified in Gene CyclerTM thermal cycler (Bio-Rad Lab.) with primer 1 and primer 4 over 30 cycles of denaturation at 94^{0}C for 60 sec, annealing at 50^{0}C for 60 sec and extension at 72^{0}C for 90 sec. A final extension of 7 min at 72^{0}C was used. The expected PCR product was 193 base pair (bp). Two microliters of PCR product were used to perform a nested PCR reaction with primer 2 and primer 3 over 30 cycles of denaturation at 94^{0}C for 30 sec, annealing at 50^{0}C for 30 sec and extension at 72^{0}C for 1 min. A final extension of 3.5 min at 72^{0}C was used. With this method, a 94-bp product could be detected on 3% MetaPhor agarose (FMC BioProducts, Rockland, ME, USA) after being treated with ethidium bromide (1 µg/ml, Promega Co.). Molecular size marker used was 100 bp DNA ladder (Promega Co). Positive control consisted of *T. gondii* DNA and negative control was digestion buffer only.

Avidin-Biotin ELISA for T. gondii antigen detection: Two pieces of one-gram sample were used. Each one gram of sample was added to the last amount of 1 ml PBS containing 0.04% phenylmethylsulfonyl fluoride (Sigma Chemical Co). Samples were then homogenized and the supernatants were collected by centrifugation. Avidin-biotin ELISA was performed as previously described (Lin and Hung, 1996). The optimal concentrations of various reagents were previously determined by checkerboard titration. One hundred µl of rabbit IgG to *T. gondii* (20 µg per ml of 0.1 M bicarbonate buffer) were put into each well of 96-well, flat-bottom, MaxiSorp microplates (Nunc, Roskilde, Denmark). Following incubation overnight at 4^{0}C, the plate was washed with 0.05% Tween 20 in PBS (PBST) by a microplate washer (Dynatech Lab., Chantilly, VA, USA). Then, 200 µl of blocking buffer [3% skim milk (Difco Lab.) in PBST] were added. The plate was incubated at 37^{0}C for 30 min and washed. After addition of 100 µl samples, the plate was incubated at 37^{0}C for 40 min. Whole *T. gondii* tachyzoite antigens were applied as a positive control. After being washed again, 100 µl biotinylated rabbit anti-*T. gondii* IgG (1 mg/ml) diluted to 1:100 in blocking solution were added. Another 40 min of incubation at 37^{0}C was performed and 100 µl of peroxidase labeled avidin (1 mg/ml, Sigma Chemical Co) diluted to 1:4,000 in blocking solution were placed in each well. The plate incubated for 40 min at 37^{0}C rinsed with PBST. One hundred microliters of substrate containing O-phenylenediamine (Sigma Chemical Co) were added. The plate was kept in the dark for 14 min at room temperature. Fifty microliters of 4M H_2SO_4 were put in to stop the reaction. Reading of the plate was at 490 nm by an ELISA reader (Dynatech Lab). Positivity was considered as the optical density value was greater than mean value of 30 negative controls plus 3 standard deviations.

Microscopic examination for T. gondii cysts: Two pieces of frozen one-gram sample from the livestock meats or internal organs, of which DNA or antigen tested positive, were ground separately using a tissue grinder. To each gram of ground tissue, 10 ml of digestion fluid [PBS containing 0.5% pepsin and 0.7% HCl acid (Sigma Chemical Co)] were added. After incubation at 37^{0}C for 2 hours with stirring, the homogenate was centrifuged 1 min at 250 xg and decanted. The sediment was then resuspended in 10 ml PBS, recentrifuged and decanted again. The resulting precipitate was mixed with 1 ml PBS and subjected to microscopic examination for *T. gondii* cysts. (Dubey, 1998).

Serum T. gondii antibody detection

Kinetic-based ELISA: Soluble whole tachyzoite antigens, 50 µl (20 µg/ml in 0.1 M bicarbonate buffer, pH 9.6) per well, were placed in the MaxiSorp™ plate (Nunc). After incubation at 4^{0}C overnight, the plate was washed with PBST by a microplate washer (Dynatech Lab). One hundred microliters of blocking solution, 3% skim milk in PBST, were then added. The plate was incubated for 40 min at 37^{0}C and was washed again. Following addition of 50 µl of 1:20 diluted dog or cat serum in blocking solution, the plate was incubated for 40 min at 37^{0}C. Negative and positive control sera were also applied. After washing, 100 µl of peroxidase-labeled either goat anti-cat IgG or rabbit anti-dog IgG antibody (Biogenesis Ltd., England, UK) at 1:8,000 dilution were added. The plate was incubated again at 37^{0}C for 40 min and washed. Following addition of 100 µl substrate, O-phenylenediamine (Sigma Chemical Co), the plate was read (at 450 nm) by an ELISA reader (Dynatech Lab.). Consequently, the reaction rate between the bound peroxidase conjugate and substrate was calculated by three absorbance data at 2-min intervals each. The absorbance values and times showed a linear relationship. Thus, the regression coefficient (slope of substrate conversion rate by enzyme) was directly proportional to the amount of antibodies existed in the serum. Samples were tested in triplicate. Positivity was considered when the mean kinetic-based ELISA value was equal to or larger than 0.02. (Lin et al., 1992). The positive samples were then subjected to immunoblotting assay.

Immunoblotting assay for P30 antibody detection: A minigel equipment (Biometra Inc., Tampa, FL, USA) was used for SDS-polyacrylamide gel electrophoresis. All the reagents used were provided by Bio-Rad Lab. *T. gondii* membrane antigens, extracted by Nonidet-P40, were mixed with reducing sample buffer containing 5% 2-mercaptoethanol. After heating for 4

min at 90^{0}C, 5 µl of the above solution, containing 12.5 µg of membrane antigens or pre-stained low molecular weight standards (Bio-Rad Lab), were subjected to 12% SDS-polyacrylamide gel. Electrophoresis was performed at 120 voltages for 1 hour. A blotting equipment (Biometra Inc) was used to transblot the gel onto a nitrocellulose paper (BA83, Schleicher & Schuell, Germany). Then, nitrocellulose paper was incubated with 1:100 diluted kinetic-based ELISA positive sera in blocking buffer containing 1% Triton X-100 (Sigma Chemical Co) for 1 hour at room temperature. Negative and positive control sera were also applied. After washing, nitrocellulose paper was incubated with 1:500 diluted peroxidase-labeled either goat anti-cat IgG or rabbit anti-dog IgG antibody (Biogenesis Ltd., England, UK) at room temperature for 1 hour and washed again. Finally, substrate, 4-chloro-1-naphthol (Sigma Chemical Co.), was added. Positivity was revealed by the formation of a 30-kD band, P30.

Statistical analysis: The seroprevalence of pets observed in this study was compared to those observed previously by us (Lin, 1998; Lin et al., 1998) using R Project for Statistical Computing.

Results

Detection of T. gondii DNA in livestock meats and internal organs by nested PCR

The appearance of a 94-bp product on agarose in one of the two pieces of one-gram sample tested was referred as positive result (Fig 1). In the pig samples, 8% of pork and 2% of pig livers were positive. Of the beef and mutton samples tested, 5% and 4% were positive respectively. In the chicken samples, *T. gondii* DNA was found in 4% of flesh and 2% of hearts, but not in gizzards or liver (Table 1). The sensitivity of the nested PCR was 25 fg DNA/ml.

Detection of T. gondii antigens in livestock meat and internal organs by avidin-biotin ELISA: No T. gondii antigens were detected in all the samples. The sensitivity of avidin-biotin ELISA was 4 ng antigen/ml.

Microscopic examination for T. gondii cysts: No *T. gondii* cysts were found in the samples from livestock meats or internal organs of which DNA was tested positive.

Detection of T. gondii antibodies in pets' sera by kinetic-based ELISA and immunoblotting:

Only sera positive in both kinetic-based ELISA and immunoblotting assay were regarded as true positive. In kinetic-based ELISA positive samples, 6 out of 7 dog sera and 10 out of 12 cat sera were also positive in immunoblotting showing a 30-kD band (Fig 2). Thus, the seroprevalence of *T. gondii* antibodies in dogs and cats were 6% and 10% correspondingly. The relative sensitivity and specificity of immunoblotting for measurement of serum IgG antibodies to P30 were 100%, 93.7% for dogs and 100%, 97.9% for cats respectively.

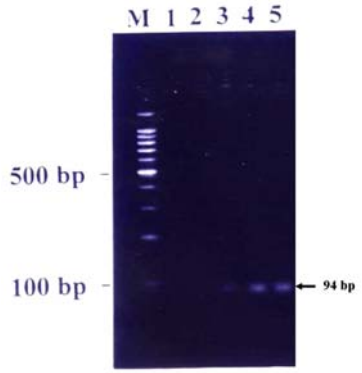

Figure 1 Nested polymerase chain reaction of pork samples after ethidium bromide staining on 2% MetaPhor agarose gel. M: molecular size markers (100 bp DNA ladder); Lane 1: digestion buffer only; Lanes 2-4: pork samples (lane 2: negative, lanes 3-4: positive); Lane 5: positive control with *T. gondii* DNA. Positive result shows the 94-bp product of *T. gondii*-specific B1 gene.

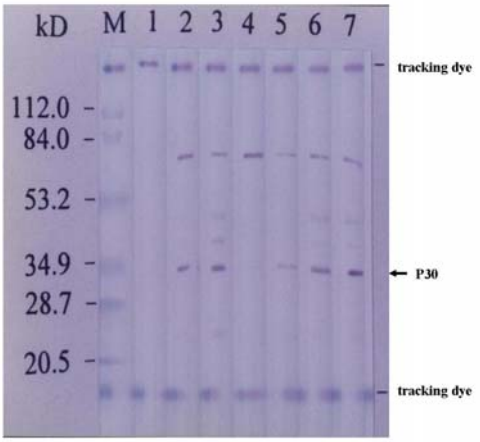

Figure 2 Immunoblotting assay for P30 antibody detection of cat serum samples. Lane 1: negative control; Lanes 2-6: cat serum samples (lanes 2, 3, 5 & 6: positive, lane 4: negative); Lane 7: positive control; M: pre-stained molecular weight standards; kD: kilodalton. Positivity is revealed by the formation of a 30-kD band, P30.

Table 1 Prevalence of *T. gondii* DNA, antigens, and cysts in livestock meats and internal organs sold at supermarkets in Taipei during 2008-2010

Method for *T. gondii* Detection	Livestock meats and Internal organs							
	Pork	Pig liver	Beef	Mutton	Chicken flesh	Gizzard	Chicken heart	Chicken liver
DNA[1]	8%	2%	5%	4%	4%	0	2%	0
Antigen[1]	0	0	0	0	0	0	0	0
Cyst[2]	0	0	0	0	0	ND[3]	0	ND

[1] N = 100; [2] Samples from the meats or internal organs of which DNA tested positive
[3] Not done

Statistical analysis

The seroprevalence of pets observed in this study is not significantly different from those reported in the year 1998 (Lin, 1998; Lin et al., 1998), which were 7.9% (n = 658) for pet dogs ($X^2 = 0.2162$, $p = 0.64$) and 14% (n = 57) for pet cats ($X^2 = 0.0453$, $p = 0.8314$).

Discussion

This is the first report on *T. gondii* in livestock meats and internal organs in Taipei. PCR is the most sensitive technique for *T. gondii* DNA detection (James et al., 1996; Cresti et al., 2001; Buchbinder et al., 2003; Hill et al., 2006[b]). In this survey, we found the prevalence of *T. gondii* DNA in pork and in pig liver to be 8% and 2% respectively. The real prevalence should be higher because the organisms might not be present in the samples cut. Recent serologic results also indicated that 10.1-28.8% of market-age pigs in Taiwan were *T. gondii* seropositive (Fan et al., 2004; Tsai et al., 2007). Nevertheless, *T. gondii* antigens were not detected in all samples and *T. gondii* cysts were not found in those DNA positive samples. It is possible that the limited sensitivity of both antigen-ELISA and microscopy contributes to the negative findings.

This report also revealed the prevalence of *T. gondii* DNA to be 5% in beef, 4% in mutton, 4% in chicken flesh, and 2% in chicken heart. No *T. gondii* antigens were detected in all samples and no cysts were found in those DNA positive samples. Nevertheless, the discovery of *T. gondii* DNA in livestock meats and internal organs indicated the presence of *T. gondii* organisms in these positive items. Currently, there are no data concerning *T. gondii* infection status of goats, chickens or imported beef in Taiwan.

By combination of both kinetic-based ELISA and immunoblotting, this study showed that 6% of pet dogs and 10% of pet cats in Taipei were *T. gondii* seropositive. Surprisingly, the seroprevalence of pets observed in this study is not significantly different from those reported in the year 1998, which were 7.9% for pet dogs (Lin, 1998) and 14% for pet cats (Lin et al., 1998). Previous study also indicated that 7% of the people in Taipei had detectable antibodies to *T. gondii* (Lin et al., 1998).

Multivariate analysis has shown that an increased risk of *T. gondii* infection is related to consuming undercooked meats, having a pet cat and poor hand hygiene (Baril et al., 1999; Lee, 2000; Dubey et al., 2005; Jones et al., 2009). It has been reported that *T. gondii* tissue cysts are killed in pork stored at -12^{0}C for 3 days (Dubey, 1988) or at 0^{0}C or below for 7 days (Hill et al., 2006[a]). In addition, cooking meat to an internal temperature of 66-67^{0}C renders tissue cysts non-viable (Dubey, 1996; Dubey et al, 2005). In Taiwan, livestock meats and organs are usually frozen before being sold in supermarkets and most people tend to cook meat before eating. However, the seroprevalence of *T. gondii* antibodies in humans and pets in Taipei is apparently higher than we expected. This discrepancy is probably due to the following reasons: 1. Although meats and internal organs are usually frozen before being put on supermarket refrigerator shelf, the freezing temperature may not be low enough and/or freezing time may not be long enough to kill *T. gondii* cysts. 2. In contrast, meats and internal organs sold in traditional markets are not frozen or treated with irradiation. 3. Furthermore, due to personal eating and cooking habits, internal temperature of the meats may not reach 67^{0}C. 4. Finally, the environments have already been contaminated by oocysts excreted by infected cats, especially stray cats.

In conclusion, in this survey of *T. gondii* in Taipei, we found the presence of DNA in livestock meats and internal organs in supermarkets and the seropositivity in cat and dog sera. These findings reflect the potential importance of livestock meats and internal organs in public health and the existence of *T. gondii* contamination in the environment. Continued education for food producer, consumers, and medical practitioners is required for further prevention and control of food-borne parasitic zoonoses.

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