**Seroprevalence of *Toxoplasma gondii* in humans and pigs in North Sulawesi, Indonesia**

Josef Tuda[a], Sri Adiani[b], Madoka Ichikawa-Seki[c], Kousuke Umeda[d], Yoshifumi Nishikawa[d,*]

[a]Department of Parasitology, Faculty of Medicine, Sam Ratulangi University, Manado 95114, Indonesia

[b]Faculty of Animal Husbandry, Sam Ratulangi University, Manado 95114, Indonesia

[c]Laboratory of Veterinary Parasitology, Faculty of Agriculture, Iwate University, 3-18-8 Ueda, Morioka 020-8550, Japan

[d]National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Nishi 2-13 Inada-cho, Obihiro 080-8555, Japan

E-mail addresses

Josef Tuda: jsbtuda@yahoo.com

Sri Adiani: sri_adiani@yahoo.de

Madoka Ichikawa-Seki: madoka@iwate-u.ac.jp

Kousuke Umeda: umedak@obihiro.ac.jp

Yoshifumi Nishikawa: nisikawa@obihiro.ac.jp

*Address correspondence to Yoshifumi Nishikawa, nisikawa@obihiro.ac.jp

**Abstract**

*Toxoplasma gondii*, an intracellular protozoan parasite, is a major public health concern throughout the world. Importantly, toxoplasmosis has several adverse effects, including neurological and ocular diseases. There are currently no data on the prevalence of *T. gondii* infection in humans or animals in North Sulawesi, although Indonesia is known to have a high seroprevalence of this parasite. In this study, the prevalence of *T. gondii* was determined in samples of humans and pigs from North Sulawesi using the latex agglutination test. In total, 856 human were sampled and 58.5% of whom were positive for *T. gondii*. Although the antibody prevalence in male and female children aged 0–9 years was less than 10%, the prevalence in individuals over 10 years old was more than 40% in both sexes, suggesting that the transmission rate of the parasite to humans is extremely high in this area. However, the overall prevalence of *T. gondii* in pigs was only 2.3%. Our study indicates a high incidence of *T. gondii* infection in humans. Therefore, a survey of the prevalence of *T. gondii* among different infection sources is required to determine the major risk factors for infection in North Sulawesi.

**Keywords:**

*Toxoplasma gondii*, toxoplasmosis, seroprevalence, Indonesia, North Sulawesi

## 1. Introduction

Toxoplasmosis caused by the obligate apicomplexan intracellular protozoan *Toxoplasma gondii* is an important cause of miscarriage or adverse fetal effects, including neurological and ocular diseases, and may also have sequelae later in the life of an infected neonate [1]. This parasite can infect most genera of warm-blooded animals and is estimated to infect 30%–50% of the human population globally [2]. The definitive hosts of *T. gondii* are members of the felid family, which shed oocysts in their feces [3]. The oocysts are remarkably stable in the environment, and are transmitted to other hosts through their inadvertent ingestion. Therefore, raw or undercooked meat from intermediate hosts, such as sheep, goats, pigs, and chickens, is potentially infectious if ingested by humans or other animals [4].

The prevalence of toxoplasmosis varies greatly between countries and even within different regions of the same country [5]. This may be attributable to the distribution of the infection source, cultural practices, or hygiene habits. The lowest seroprevalence (less than 1%) is found in some countries in the Far East, whereas the highest (more than 90%) occurs in some parts of European and South American countries [6]. The prevalence of *T. gondii* in Indonesia, one of the strongly affected countries, is estimated to be around 50%. Although several studies have reported a high prevalence of *T. gondii* in different regions of Indonesia, there has been no survey of its occurrence in North Sulawesi. In this study, we investigated the seroprevalence of *T. gondii* in humans and pigs in North Sulawesi to supplement the existing epidemiological data for *T. gondii* in Indonesia.

## 2. Materials and methods

### *2.1. Sample population*

Humans were sampled in Manado, Bolmong, Sangihe Talaud, Minahasa, Bitung, and others (north maluku, gorontalo) in North Sulawesi, Indonesia. Serum samples (N = 856) were randomly collected between January 2016 and March 2016 in the 1st sample collection. In the 2nd sample collection, 156 human aged 0–20 years were sampled in Bitung. The details of the various groups of humans and the geographic locations of the collection sites are shown in Tables 1 and 2, respectively, and in Figure 1. The study was reviewed and approved by the Institutional Review Board of the Faculty of Medicine, Sam Ratulangi University, Manado, Indonesia (03/KEP/I/2016). Written informed consent was obtained from all the participants.

Pigs were sampled in Manado, Tomohon, Minahasa, and Bitung in North Sulawesi, Indonesia. The serum samples were collected in June 2015. The details of the geographic locations of the collection sites are shown in Table 3 and Figure 1.

### *2.2. Blood collection*

Venous blood (1 ml) was drawn from the cubital veins of humans and from the jugular veins of pigs. The plasma was isolated from each blood sample by centrifugation at 2,000 ×*g* for 10 min and stored at –30 °C until analysis.

### *2.3. Latex agglutination test (LAT)*

To detect *T. gondii* infections, the sera were tested with LAT using Toxocheck-MT (Eiken Chemical, Tokyo, Japan), according to the manufacturer's instructions. Samples were considered positive when agglutination was observed at a

dilution of 1:32. The antibody reactivity to *Toxoplasma* antigen (rated from 0 to 3) was estimated by the sedimentation pattern, according to the manufacturer's instructions.

## 3. Results

Human serum samples from North Sulawesi, Indonesia, were tested for anti-*Toxoplasma* antibodies. Of the 856 human sera in the 1st sample collection, 501 (58.5%) were seropositive for *T. gondii* (Table 2). According to the sampling location, the prevalence rates were 221/392 (56.4%) for Manado, 14/25 (56.0%) for Bolmong, 15/28 (53.6%) for Sangihe Talaud, 72/115 (62.6%) for Minahasa, 171/282 (60.6%) for Bitung, and 8/14 (57.1%) for others (north maluku, gorontalo). The prevalence for *T. gondii* did not differ significantly among the sampling sites.

To analyze the age-related prevalence of *T. gondii*, the sera of 156 humans aged 0–20 years from Bitung, obtained with the 2nd sample collection and 856 humans in the 1st sample collection, were analyzed (Fig. 2). Although there was no statistically significant difference in the prevalence for *T. gondii* between male and female individuals, the *T. gondii*-positive rate increased significantly after 10 years of age (Fig. 2A). Serum antibody reactivity to *Toxoplasma* antigen also tended to increase with age (Fig. 2B).

Because the infection of humans by *T. gondii* is mediated by the meat of the infected animals, the seroprevalence of this parasite in pigs was examined in North Sulawesi (Table 3). The prevalence rates were 0/11 (0%) for Manado, 1/76 (1.3%) for Tomohon, 4/71 (5.6%) for Minahasa, and 2/152 (1.3%) for Bitung. The total prevalence of *T. gondii* across these locations was 7/310 (2.3%).

## 4. Discussion

This study is the first to report the prevalence of *T. gondii* in North Sulawesi, Indonesia. The overall prevalence of *T. gondii* in humans was 58.5%, which is similar to that reported in previous studies of West Java (Surabaya, 58% [7], Jakarta, 70% [8]), and East Java (Sidoarjo, 64% [9]). In a study undertaken in the Malili area of South Sulawesi, 62% of humans were seropositive for *T. gondii* [10], and a high seroprevalence was also reported in West Papua (Irian Jaya, 65% [11]). *Toxoplasma gondii* infection has also been observed in humans in other parts of Indonesia, with rates of 30.8% in Surakarta (Central Java [12]), 3% in West Kalimantan (Borneo [13]), 9% in North Sumatra [14], and 27.1% in Lindu Valley (Central Sulawesi [15]). Therefore, compared with the nationwide prevalence of *T. gondii* in Indonesia, the prevalence of human infections in North Sulawesi is relatively high.

Our study shows that *T. gondii* infections in human in North Sulawesi are associated with age, with their prevalence increasing after 10 years of age. Similar results have been reported from East Java, in both Surabaya [7] and Sidoarjo [9], and in the Malili area of South Sulawesi [10]. In northeast Bali, the prevalence of infection among children and teenagers is 3.1% [16]. Although one study reported that the prevalence of anti-*T. gondii* antibodies does not increase with age in Irian Jaya (West Papua) [17], age is still a risk factor for *T. gondii* infection in Indonesia.

In this study, children aged 0–1 years were not seropositive for *T. gondii*, so the vertical transmission of the infection is unlikely. Therefore, the transmission of the parasite from food and the environment must be considered. Although most (> 80%)

Indonesians are Muslim [18], most people in North Sulawesi are protestant Christians and Chinese. Therefore, pigs are an important food animal in North Sulawesi. However, in this study, the overall prevalence of *T. gondii* in pigs was only 2.3%. Pork provided from intensive farm is now popular in North Sulawesi while non-intensive local farm provides pork in rural area. The pig samples were collected from intensive farming systems, so this low seropositive rate could be attributable high standards of veterinary care, close animal observation, and good biosecurity measures. However, previous studies have also shown relatively low prevalence rates of *T. gondii* among pigs in Indonesia, with 3.6% in Bandar Lampung (Sumatra) and 9.2% in Ujung Pandang (Sulawesi) [19]. We also found that 14.6% of pigs were seropositive for *T. gondii* in West Java [20]. Therefore, because the prevalence of *T. gondii* is much higher in humans than in pigs, pigs may not be a major infection source for human infection.

Several studies have reported the prevalence of *T. gondii* in other animal species in Indonesia. In cattle, we detected *T. gondii* seropositive rates of 14/94 (14.9%) in Manado in 2006 and 7.4% in West Java in 2015 [20]. *Toxoplasma gondii* infections were also observed in cattle in Lampung Province, southern Sumatra (9.9%) [21]. More importantly, the prevalence of *T. gondii* in goats is higher than in other animals, with 47.5% in Lampung Province [21] and 61% in South Kalimantan [22]. Therefore, goat meat may be a significant source of *Toxoplasma* infection among humans in Indonesia.

Cat feces containing *Toxoplasma* oocysts are also a source of *T. gondii* infection in livestock animals. More cat-owning families had positive *T. gondii* antibody titers than families without cats in Lindu Valley, Central Sulawesi [15]. Although there are no recent studies on *Toxoplasma* infection in cats from Indonesia, previous report in 1976 showed that 41% of cats were seropositive for *T. gondii* in South Kalimantan [22],

suggesting that soil contamination with *T. gondii* oocysts must be considered. The prevalence of *T. gondii* in free-ranging chickens is a good indicator of the prevalence of their oocysts in the soil, and 24.4% chickens are infected with *T. gondii* in Indonesia [23]. People in North Sulawesi also eat chicken, duck, bird, cat, dog and rat. Therefore, our next study will survey the prevalence of *T. gondii* in goats, cats, and chickens to determine whether they are major infection sources for human infection in North Sulawesi.

Toxoplasmosis is a common and serious central nervous system infection in patients infected with HIV [24]. In Indonesia, toxoplasmosis is always considered in HIV-infected patients with meningitis [25]. Toxoplasmosis is also associated with abortion and chorioretinitis, and these associations have been confirmed in Indonesia [11]. Because *T. gondii* is associated with several pathologies, epidemiological and ecological studies are required to confirm the causal relationship between these factors. The risk factors for toxoplasmosis in Indonesia must be investigated in more detail in future studies to understand its correlation with other diseases including epilepsy, obsessive compulsive disorder, and congenital abnormalities.

## 5. Conclusion

This study provides valuable data on the high prevalence of *T. gondii* in humans in North Sulawesi, Indonesia, which will contribute to the development of future prevention and control strategies for toxoplasmosis. To determine the major risk factors for *T. gondii* infection in North Sulawesi, a survey of the prevalence of the parasite in different potential sources of infection is required. Additionally, isolation of viable

*Toxoplasma* from food animals and cats should be important for further epidemiology of toxoplasmosis in Indonesia. More research and effort by governmental and nongovernmental authorities are also required to minimize the rate of infection.

**Acknowledgments**

The authors would like to thank Dr. Ryuichiro Maeda (Obihiro University of Agriculture and Veterinary Medicine), Dr. Yutaka Suzuki (The University of Tokyo), and Dr. Junya Yamagishi (Hokkaido University) for their valuable help in coordinating the experiments performed in Indonesia. This work was supported by a Grant-in-Aid for Scientific Research (B) from MEXT KAKENHI (grant number 26304037).

**Conflict of interest statement**

The authors declare that they have no conflicts of interest.

**References**


[1] I.M. Rodrigues, T.L. Costa, J.B. Avelar, W.N. Amaral, A.M. Castro, M.M. Avelino, Assessment of laboratory methods used in the diagnosis of congenital toxoplasmosis after maternal treatment with spiramycin in pregnancy, BMC Infect. Dis. 14 (2014) 349.

[2] J. Flegr, J. Prandota, M. Sovičková, Z.H. Israili, Toxoplasmosis--a global threat. Correlation of latent toxoplasmosis with specific disease burden in a set of 88 countries, PLoS One. 9 (2014) e90203.

[3] E.F. Torrey, R.H. Yolken, *Toxoplasma* oocysts as a public health problem, Trends Parasitol. 29 (2013) 380–384.

[4] J.P. Dubey, Toxoplasmosis of animals and humans. 2nd ed., Boca Raton: CRC Press Inc, (2010) 1–313.

[5] S.A. Elmore, J.L. Jones, P.A. Conrad, S. Patton, D.S. Lindsay, J.P. Dubey, *Toxoplasma gondii*: epidemiology, feline clinical aspects, and prevention, Trends Parasitol. 26 (2010) 190-196.

[6] J. Flegr, Influence of latent *Toxoplasma* infection on human personality, physiology and morphology: pros and cons of the *Toxoplasma*-human model in studying the manipulation hypothesis, J. Exp. Biol. 216 (2013) 127–133.

[7] E. Konishi, Y. Houki, K. Harano, R.S. Mibawani, D. Marsudi, S. Alibasah, Y.P. Dachlan, High prevalence of antibody to *Toxoplasma gondii* among humans in Surabaya, Indonesia, Jpn. J. Infect. Dis. 53 (2000) 238–241.

[8] A. Terazawa, R. Muljono, L. Susanto, S.S. Margono, E. Konishi, High *Toxoplasma* antibody prevalence among inhabitants in Jakarta, Indonesia, Jpn. J. Infect. Dis. 56 (2003) 107–109.

[9] S. Uga, K. Ono, N. Kataoka, H. Hasan, Seroepidemiology of five major zoonotic parasite infections in inhabitants of Sidoarjo, East Java, Indonesia, Southeast Asian J. Trop. Med. Public Health. 27 (1996) 556–561.

[10] W.P. Carney, J.H. Cross, S.W. Joseph, P.F. Van Peenen, D. Russell, J. Sulianti Saroso, Serological study of amoebiasis and toxoplasmosis in the Malili area, South Sulawesi, Indonesia, Southeast Asian J. Trop. Med. Public Health 9 (1987) 471–479.

[11] S. Gandahusada, Study on the prevalence of toxoplasmosis in Indonesia: a review, Southeast Asian J. Trop. Med. Public Health. 22 (1991) 93–98.

[12] A.A. Prasetyo, R. Ariapramuda, E.A. Kindi, P. Dirgahayu, Y. Sari, R. Dharmawan, S. Kageyama, Men having sex with men in Surakarta, Indonesia: demographics, behavioral characteristics and prevalence of blood borne pathogens, Southeast Asian J. Trop. Med. Public Health. 45 (2014) 1032–1047.

[13] J.H. Cross, M.D. Clarke, W.C. Cole, J.C. Lien, F. Partono, Djakaria, A. Joesoef, S. Oemijati, Parasitic infections in humans in West Kalimantan (Borneo), Indonesia, Trop. Geogr. Med. 28 (1976) 121–130.

[14] J.H. Cross, M.D. Clarke, W.C. Cole, J.C. Lien, F. Partono, A. Joesoef, E.H. Kosin, Parasitology survey in northern Sumatra, Indonesia, J. Trop. Med. Hyg. 79 (1976) 123–131.

[15] M.D. Clarke, J.H. Cross, W.P. Carney, P. Hadidjaja, A. Joesoef, J. Putrali, Sri Oemijati, Serological study of amebiasis and toxoplasmosis in the Lindu Valley, Central Sulawesi, Indonesia, Trop. Geogr. Med. 27 (1975) 274–278.

[16] B.B. Chome, R. Kasten, C. Adams, D. Lambillotte, J. Theis, R. Goldsmith, J. Koss, C. Chioino, D.P. Widjana, P. Sutisna, Serosurvey of some major zoonotic

infections in children and teenagers in Bali, Indonesia, Southeast Asian J. Trop. Med. Public Health 24 (1993) 321–326.

[17] S. Gandahusada, S. Endardjo, *Toxoplasma* antibodies in Obano, Irian Jaya, Indonesia, Southeast Asian J. Trop. Med. Public Health. 11 (1980) 276–279.

[18] Pew Research Center's Forum on Religion & Public Life, The Global Religious Landscape. (2012) http://www.pewforum.org/files/2014/01/global-religion-full.pdf

[19] I. Inoue, C.S. Leow, D. Husin, K. Matsuo, P. Darmani, A survey of *Toxoplasma gondii* antibodies in pigs in Indonesia, Southeast Asian J. Trop. Med. Public Health. 32 (2001) 38–40.

[20] M. Ichikawa-Seki, A. Guswanto, P. Allamanda, E.S. Mariamah, P.E. Wibowo, I. Igarashi, Y. Nishikawa, Seroprevalence of antibody to TgGRA7 antigen of *Toxoplasma gondii* in livestock animals from Western Java, Indonesia, Parasitol. Int. 64 (2015) 484–486.

[21] K. Matsuo, D. Husin, A survey of *Toxoplasma gondii* antibodies in goats and cattle in Lampung province, Indonesia, Southeast Asian J. Trop. Med. Public Health. 27 (1996) 554–555.

[22] P.T. Durfee, J.H. Cross, Rustam, Susanto. Toxoplasmosis in man and animals in South Kalimantan (Borneo), Indonesia, Am. J. Trop. Med. Hyg. 25 (1976) 42–47.

[23] J.P. Dubey, L.T. Huong, B.W. Lawson, D.T. Subekti, P. Tassi, W. Cabaj, N. Sundar, G.V. Velmurugan, O.C. Kwok, C. Su, Seroprevalence and isolation of *Toxoplasma gondii* from free-range chickens in Ghana, Indonesia, Italy, Poland, and Vietnam, J. Parasitol. 94 (2008) 68–71.

[24] V. Nissapatorn, C. Lee, K.F. Quek, C.L. Leong, R. Mahmud, K.A. Abdullah, Toxoplasmosis in HIV/AIDS patients: a current situation, Jpn. J. Infect. Dis. 57

(2004) 160–165.

[25] A.R. Ganiem, S. Dian, A. Indriati, L. Chaidir, R. Wisaksana, P. Sturm, W. Melchers, A. van der Ven, I. Parwati, R. van Crevel, Cerebral toxoplasmosis mimicking subacute meningitis in HIV-infected patients; a cohort study from Indonesia, PLoS Negl. Trop. Dis. 7 (2013) e1994.

## Figure legend

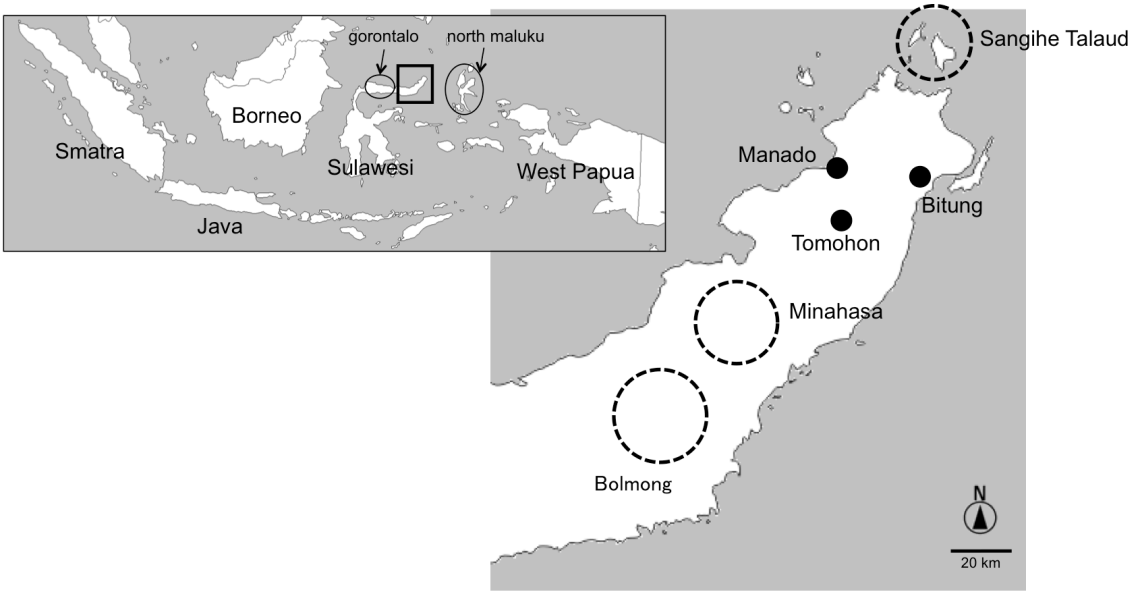


Fig. 1. Tuda et al.

**Fig. 1.** Geographic distribution of the sampling sites used in this study. The names of the districts/cities are shown on the map.

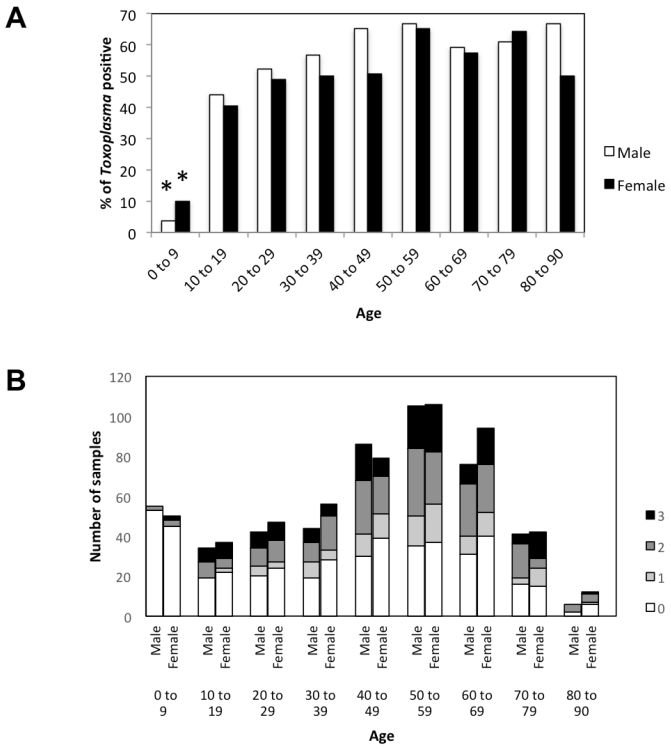


Fig. 2. Tuda et al.

**Fig. 2.** Age-related prevalence of antibodies against *T. gondii* (A) and the distributions of antibody titers (B) in male and female individuals sampled in the 1st and 2nd collections. Ages are grouped in 10-year increments. The antibody reactivity to *Toxoplasma* antigen (rated from 0 to 3) by the sedimentation pattern was shown in (B). *A statistically significant difference in the prevalence of *T. gondii* antibodies was observed between the 0–9-year and 10–19-year age groups with a $\chi^2$ test ($P < 0.05$).

**Table 1**

Age and sex data for the 1st and 2nd sample collections

| Age (years) | Number of individuals | | Total |
|---|---|---|---|
| | Male | Female | |
| 0 to 9 | 55 | 50 | 105 |
| 10 to 19 | 34 | 37 | 71 |
| 20 to 29 | 42 | 47 | 89 |
| 30 to 39 | 44 | 56 | 100 |
| 40 to 49 | 86 | 79 | 165 |
| 50 to 59 | 105 | 106 | 211 |
| 60 to 69 | 76 | 94 | 170 |
| 70 to 79 | 41 | 42 | 83 |
| 80 to 90 | 6 | 12 | 18 |

**Table 2**

Seroprevalence of *T. gondii* in humans sampled in the 1st sample collection at different sites in North Sulawesi, Indonesia.

| Place | Sample number | | | Number of *Toxoplasma* positive sample | | | % of *Toxoplasma* positive | | |
|---|---|---|---|---|---|---|---|---|---|
| | Male | Female | Total | Male | Female | Total | Male | Female | Total |
| Manado city | 190 | 202 | 392 | 113 | 108 | 221 | 51.1 | 48.9 | 56.4 |
| Bolmong (Bolaang Mongondow district) | 18 | 7 | 25 | 10 | 4 | 14 | 71.4 | 28.6 | 56.0 |
| Sangihe Talaud district | 13 | 15 | 28 | 7 | 8 | 15 | 46.7 | 53.3 | 53.6 |
| Minahasa district | 62 | 53 | 115 | 34 | 38 | 72 | 47.2 | 52.8 | 62.6 |
| Bitung city | 123 | 161 | 282 | 83 | 88 | 171 | 48.5 | 51.5 | 60.6 |
| Others (north maluku, gorontalo) | 7 | 7 | 14 | 3 | 5 | 8 | 37.5 | 62.5 | 57.1 |
| Total | 413 | 445 | 856 | 250 | 251 | 501 | 49.9 | 50.1 | 58.5 |

**Table 3**

Seroprevalence of *T. gondii* in pigs at different sites in North Sulawesi, Indonesia.

| Place | Sample number | Number of *Toxoplasma* positive sample | % of *Toxoplasma* positive |
|---|---|---|---|
| Manado city | 11 | 0 | 0 |
| Tomohon city | 76 | 1 | 1.3 |
| Minahasa district | 71 | 4 | 5.6 |
| Bitung city | 152 | 2 | 1.3 |
| Total | 310 | 7 | 2.3 |