Jurnal Kedokteran Hewan
P-ISSN : 1978-225X; E-ISSN : 2502-5600
December 2021, 15(4):140-146
DOI: https://doi.org/10.21157/j.ked.hewan.v15i4.21716

COMPARISON OF DIFFERENT COMMERCIAL SEROLOGY KITS FOR THE DETERMINATION OF SEROPOSITIVE TOXOPLASMOSIS IN CATTLE IN INDONESIA

Didik T Subekti[1]*, Sisca Valinata[2], and Sulinawati Fong[2]
[1]Indonesian Research Center for Veterinary Sciences, Bogor, Indonesia
[2]Lampung Disease Investigation Center, Bandar Lampung, Indonesia
*Corresponding author: didiktulus@pertanian.go.id

ABSTRACT

This research aims to explain the evaluation of the differences in four commercial kits for the detection of serological toxoplasmosis used in Indonesia. The results of the study found that the toxoplasmosis seropositivity determined by the four commercial kits showed a significant difference (P<0.05). Seropositive toxoplasmosis obtained using Pastorex, Toxotest, IDScreen, and Toxo Ab were 35.12%, 60.12%, 26.19%, and 10.12% respectively. IDScreen had a good agreement with Toxo Ab (Gwet's AC_1= 0.623) and a moderate agreement with Pastorex (Gwet's AC_1= 0.494-0.511). Toxotest had a low agreement with three commercial kits (Gwet's AC_1= <0.2) but had a moderate agreement with western blotting (WB) and modified agglutination test (MAT) (Gwet's AC_1= 0.458-0.557).

Key words: cattle, ELISA, seroprevalence, toxoplasmosis

ABSTRAK

Penelitian ini bertujuan untuk menjelaskan evaluasi perbedaan empat kit komersial untuk deteksi serologi toksoplasmosis yang digunakan di Indonesia. Hasil penelitian menemukan bahwa seropositif toksoplasmosis yang ditentukan oleh keempat kit komersial menunjukkan perbedaan yang signifikan (P<0,05). Toksoplasmosis seropositif yang diperoleh dengan menggunakan Pastorex, Toxotest, ID Screen, dan Toxo Ab berturut-turut adalah 35,12%, 60,12%, 26,19%, dan 10,12%. IDScreen memiliki kesepakatan yang baik dengan Toxo Ab (Gwet's AC_1= 0,623) dan kesepakatan moderat dengan Pastorex (Gwet's AC_1= 0,494-0,511). Toxotest memiliki kesepakatan yang rendah dengan tiga kit komersial (Gwet's AC_1= <0,2) tetapi memiliki kesepakatan yang moderat dengan western blotting (WB) dan modified agglutination test (MAT) (Gwet's AC_1= 0,458-0,557).

Kata kunci: sapi, ELISA, seroprevalensi, toksoplasmosis

INTRODUCTION

Toxoplasmosis is a zoonotic disease found all over the world. In Asia, the cumulative cases of toxoplasmosis in humans were reported to reach 16.4% (Molan *et al*., 2019). Toxoplasmosis in humans can cause miscarriage, chorioretinitis and uveitis, encephalitis and cerebral calcification, hydrocephalus, and birth defects (Montoya, 2002; Capobiango *et al*., 2016). Cases of toxoplasmosis in Indonesia are reported to exceed 60% in people of the children bearing-age (Terazawa *et al*., 2003) and 19% in uveitis cases (Kurniawan *et al*., 2020).

Bovine toxoplasmosis has great potential to be a source of transmission of toxoplasmosis to humans. Bovine toxoplasmosis has been widely reported in various countries such as 13.3% in Sudan (Elfahal *et al*., 2013), 18.9% in Romania (Dubey *et al*., 2014), 17.38% in France (Blaga *et al*., 2019), and 2.3% in China (Yu *et al*., 2007. Differences in the prevalence of toxoplasmosis in these countries are likely related to the location of the country, the climate, the grazing or cultivation system, the age or history of direct or indirect contact with cats as well as differences in serological assay methods (Gamble *et al*., 2005; Steinparzer *et al*., 2014; Tagwireyi *et al*., 2019).

The seroprevalence of toxoplasmosis in cattle in various regions in Indonesia is also very diverse. Wates Disease Investigation Center (DIC) which uses the latex agglutination test (LAT), namely the Pastorex kit, reported a 49.47% toxoplasmosis seroprevalence in cattle in Central Java (Wates DIC, 2018). Toxoplasmosis seroprevalence in cattle in Lampung was reported to have reached 88.23% using modified agglutination test (MAT) (Wulandari *et al*., 2019). The seroprevalence of toxoplasmosis in cattle in South Kalimantan was reported to be 9.09% by Banjarbaru DIC using enzyme-linked immunosorbent assay (ELISA) IDScreen kit (Banjarbaru DIC, 2019). Meanwhile, a 47.75% serorevalence of toxoplasmosis in cattle in West Sumatra was reported by DIC Bukittinggi using ELISA Toxotest kits (Bukittinggi DIC, 2019).

The difference in toxoplasmosis seroprevalence is likely to be influenced by variations in the commercial serology kits used. Four commercial serology test kits for toxoplasmosis diagnosis are known to circulate and in use in Indonesia. Different commercial kits have been used by eight national DIC that routinely conduct surveillance. Data on toxoplasmosis seroprevalence obtained by each DIC are a data source for the national toxoplasmosis mapping in Indonesia.

So far, the comparison of the use of commercial serological test kits circulating in Indonesia has never been evaluated. This manuscript will evaluate the agreement among the four commercial kits applied in Indonesia. It is indispensable to ensure the diversity of toxoplasmosis information in cattle in Indonesia is free from bias caused by test kits.

Revised 07-08-2021, Accepted 23-09-2021

MATERIALS AND METHODS

Serum Samples

A total of 184 serum samples used in this study were provided by DIC Lampung from their bank serum collection. The samples previously came from the serum archive of the annual surveillance that had been carried out by DIC Lampung in its working area. Routine surveillance carried out by each DIC to monitor the animal health status in its working area is a mandatory task for all DICs in Indonesia, including DIC Lampung. Each serum sample from bank serum collection was tested using four commercial serological assays as described in Figure 1.

The agglutination assay was conducted using Pastorex (BioRad, France), a commercial LAT kit. A comparative agglutination assay was performed using an in-house MAT. The test procedure using Pastorex followed the instructions described by the manufacturer. The MAT followed the procedures described by Dubey and Desmont (1987) and Al-Adhami *et al.* (2016). In brief, the serum was diluted 1:20 with phosphate-buffered saline (PBS) pH 7.2 homogeneously. A volume of 25 uL of each diluted serum was poured into microwell (U-shaped bottom) along with 25 uL of inactivated tachyzoite suspension and was homogenized. The microwell plate was incubated at 4-8° C overnight. The microwell plate was read visually and the reaction result was declared to be negative if a pink button was formed at the bottom of the microwell and was declared as positive if it was dispersed at the bottom of the microwell. A sample was declared as seronegative if a pink button was formed at a dilution of ≤ 1:20.

Enzyme-Linked Immunosorbent Assay (ELISA)

The serology assay with ELISA was conducted using three commercial kits, Checkit Toxotest (IDEXX, Switzerland), Toxo Ab (Cusabio, China), and IDScreen (IDVet, France). The ELISA procedures for each kit were carried out following the instructions described by their respective manufacturers.

Western Blotting

Toxoplasma gondii protein was obtained by sonication using a Q500 Sonicator (QSonica, USA). Sonication was performed with a 10:0.5 pulse, 80% AMP with a 5-time repeat cycle, and then centrifuged using Allegra X-15R (Beckman Coulter, USA) at 5000 rpm, 4° C for 20 minutes. The supernatant was separated as soluble toxoplasma antigens (STA) and was quantified using the Bradford method using Quick Start™ Bradford Protein Assay (BioRad, USA). Electrophoresis was performed using 12% Mini Protean® TGX™ Precast gel (BioRad, USA) with a Spectra™ Broad Range Multicolor protein ladder (Thermo Scientific, USA) as a protein marker. Electrophoresis was carried out using Mini Protean (BioRad, USA) at 150 volts for 45-50 minutes which was then transferred to the nitrocellulose membrane using the Trans-Blot® Turbo™ Systems (BioRad, USA).

The nitrocellulose membrane was then stained using the Pierce™ Reversible Protein Stain kit (Thermo Scientific, USA) following the instructions described by the manufacturer. The nitrocellulose strips were then cut and cleaned to remove the dye. All the pieces of nitrocellulose membrane strips were blocked with a blocking buffer containing PBS pH 7.2, Tween-20 0.05% (Sigma-Aldrich, USA), and bovine serum albumin (BSA) 0.5% (Sigma-Aldrich., USA) then incubated for one hour at 30° C. The nitrocellulose membranes were washed again using a washing buffer containing PBS with Tween-20 0.05%. Each strip of nitrocellulose membrane was reacted with each serum sample at a dilution of 1:200 using PBS with Tween-20 and then incubated for an hour at room temperature. Each strip of nitrocellulose was washed with a washing buffer and then reacted with an anti-Bovine IgG conjugate-HRP (Sigma-Aldrich, USA) at a dilution of 1:10,000 then incubated for an hour at room

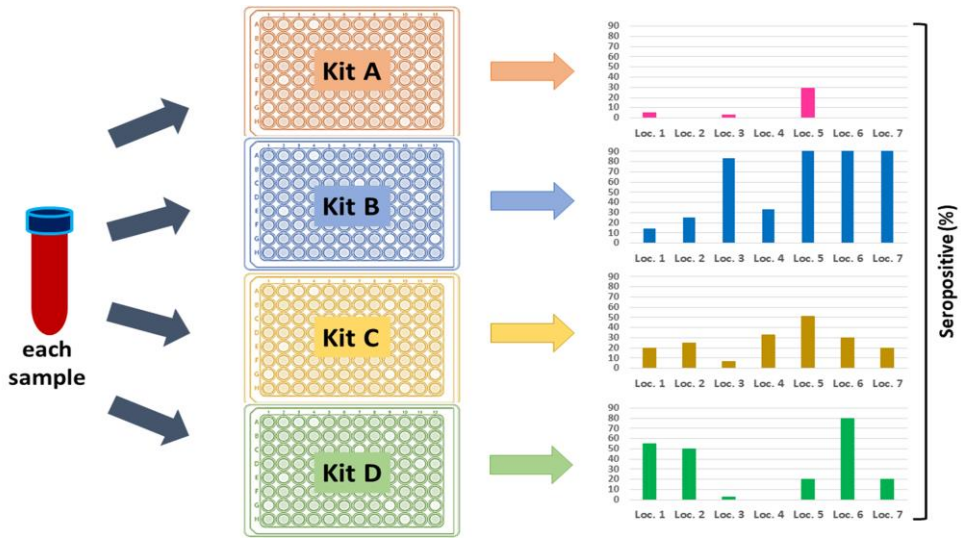

Figure 1. Flowchart of sample testing using four commercial kits in each location of sample origin

temperature. Finally, the nitrocellulose strips were washed again as before, then visualized with σ dianisidine (ODN) 0.03% (Sigma-Aldrich, USA) and stopped by rinsing with distilled water. A seropositive serum reveals brownish-orange bands on the nitrocellulose membrane.

Data Analysis

The data were analyzed using analysis of variance followed by Tukey's test. Inter-reliability analysis between commercial kits based on coefficients from Cohen's kappa, Scott's pi, Gwet's AC_1, Krippendorff's alpha, and Brennan-Prediger was conducted using AgreeStat360 (Gwet, 2016).

RESULTS AND DISCUSSION

Differences in Seropositive Determination by Four Commercial Test Kits

The difference in toxoplasmosis seroprevalence is influenced by many factors, including the environment/geography (Burells *et al*., 2018; Blaga *et al*., 2019), raising system (Fajardo *et al*., 2013, Bărburaş *et al*., 2019), as well as differences in serological assay methods (Gamble *et al*., 2005; Steinparzer *et al*., 2014). In this study, the causative factors of these differences were eliminated by testing each serum in parallel using four commercial serological assay kits. Serum samples tested using all four commercial kits produced a diversity of toxoplasmosis seropositivity in cattle (Figure 2). The highest seropositive toxoplasmosis was detected using the Toxotest kit (60.12%) and it was significantly different (P<0.01) from the Toxo Ab kit that detected the lowest seropositive toxoplasmosis (10.12%). Toxotest kit was also significantly different (P<0.05) compared to the IDScreen kit that detected a 26.19% seropositive toxoplasmosis. Seropositive toxoplasmosis with Pastorex kits showed no significant difference (P>0.05) compared to the other three commercial kits. Similarly, seropositive toxoplasmosis using the IDScreen kit also did not show significant difference (P>0.05) from that of the Toxo Ab kit.

These results prove that the four commercial kits have very different sensitivities. Therefore, its use will greatly affect the determination of the seropositive and seronegative status of the samples. This will result in biased information on the seroprevalence of Toxoplasmosis, especially if each region uses different commercial kits.

Differences in establishing seropositive and seronegative toxoplasmosis among the four commercial kits resulted in the diversity of toxoplasmosis seroprevalence in cattle in each region (Figure 3 and Table 1). The Toxotest and IDScreen kits detected the same or almost identical toxoplasmosis seroprevalence in South Sumatra Province, South Bengkulu District, and East Lampung District. The Pastorex and Toxo Ab kits both failed to detect seropositive toxoplasmosis in East Lampung District. In North Lampung District, Pastorex and Toxo Ab kits both detected a toxoplasmosis seroprevalence in cattle of 3.3% while the IDScreen kit detected 6.7%. The Toxotest kit was the only commercial kit that detected toxoplasmosis seroprevalence in cattle above 60% in the 4 regions (57.14%).

Simulation of the difference in seroprevalence due to the use of different commercial kits (Figure 3) requires us to determine the highest agreement among the various diagnostic kits that are available. This is aimed for reducing bias and maintaining homogeneity of information on diagnostic test results nationally in

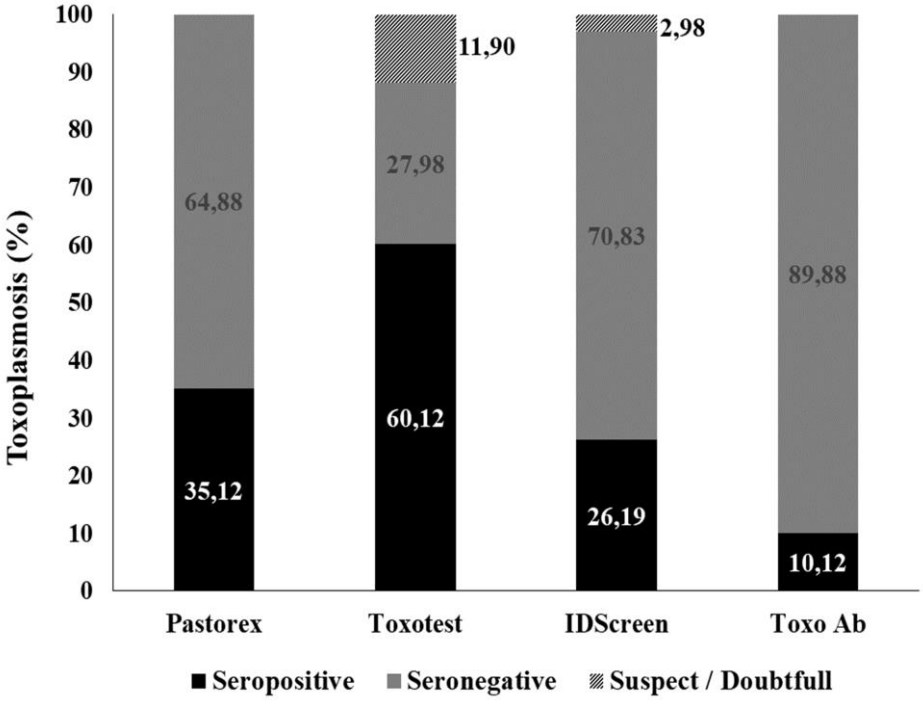

Figure 2. Distribution of seropositive toxoplasmosis in cattle based on each commercial serological test kit

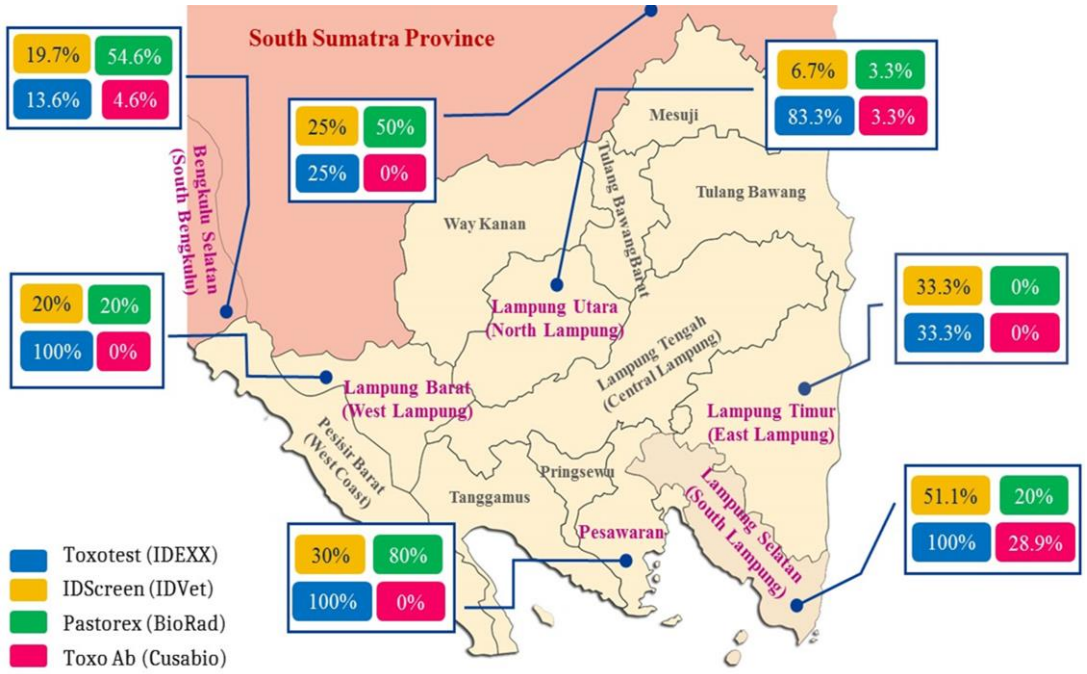

Figure 3. Diversity of toxoplasmosis seroprevalence in each location based on the test results from four different commercial kits

Table 1. The distribution of seropositive, seronegative, and dubious toxoplasmosis results in each location

Sample Origin	Seropositive (%)				Seronegative (%)				Suspect/Doubtfull (%)			
	Pastorex	Toxotest	ID Screen	Toxo Ab	Pastorex	Toxotest	ID Screen	Toxo Ab	Pastorex	Toxotest	ID Screen	Toxo Ab
Liwa, West Lampung	20	100	20	0	80	0	80	100	0	0	0	0
Malangsari, South Lampung	0	100	60	0	100	0	40	100	0	0	0	0
Pesawaran	80	100	30	0	20	0	70	100	0	0	0	0
Candipuro, South Lampung	22.5	100	50	32.5	77,5	0	50	67.5	0	0	0	0
Abung Timur, North Lampung	4	100	4	4	96	0	96	96	0	0	0	0
Sungkai Utara, North Lampung	20	0	0	0	80	100	100	100	0	0	0	0
Rama Puja, East Lampung	0	33.3	33.3	0	100	66.67	66.7	100	0	0	0	0
Manna, South Bengkulu	54.6	13.6	19.7	4.6	45.5	57.58	72.7	95.5	0	28.8	7.6	0
Musi Banyuasin, South Sumatra	50	25	25	0	50	50	75	100	0	25	0	0

various regions. Compatibility testing among various commercial kits is helpful for classifying commercial kits that have similar diagnostic sensitivity.

Agreement of Serological Test Results Among the Four Commercial Kits and Comparison with In-house MAT and WB

The commercial kits were also compared for reliability using modified agglutination test and western blotting which are more sensitive methods. MAT has long been reported to have better sensitivity in detecting toxoplasmosis in cattle (Dubey *et al*., 1985). Western blotting (WB) has high sensitivity and specificity, so it is used as a reference and confirmation test to evaluate the reliability of other toxoplasmosis serology assays (Garcia *et al*., 2008; Gu *et al*., 2015). WB is also claimed to be more reliable than MAT and ELISA in detecting toxoplasmosis in pigs (Al-Adhami and Gajadhar, 2014).

The IDScreen kit with Toxo Ab showed a good agreement (Gwet's AC_1= 0.623) according to the benchmark reported by Altman (1991) as seen in (Table 2). The Pastorex kit showed moderate agreement with the ID Screen kit and Toxo Ab (Gwet's AC_1= 0.494-0.511). However, the Toxotest kit showed a poor agreement with the other three commercial kits (Gwet's AC_1= <0.2). The Toxotest kit demonstrated a moderate agreement with the WB (Gwet's AC_1= 0.458) and MAT (Gwet's AC_1= 0.557). WB and MAT are two serological tests that are routinely applied as confirmatory tests for toxoplasmosis in DIC Lampung. In contrast, the other three commercial kits had a poor agreement with both WB and MAT (Gwet's AC_1= <0.2).

The four commercial kits evaluated in this study can be separated into two groups based on the agreement of the results. The first group consists of Toxo Ab, IDScreen, and Pastorex kits while the other

group is the Toxotest kit. The Toxo Ab and IDScreen kits had a good agreement (Gwet's AC_1= 0.623), so they could be applied together or are complementary. Pastorex's agglutination kit which had an intermediate agreement with the Toxo Ab and IDScreen kits (Gwet's AC_1= 0.494-0.511) can be considered as a replacement alternative. The Toxotest kit had a poor agreement with the other three commercial kits (Gwet's AC_1= ≤0.2), so it cannot be applied together with the other three kits. In general, these results contradict the conclusion of Steinparzer *et al*. (2014) who reported that commercial ELISA kits for the detection of toxoplasmosis have an excellent or almost perfect agreement with each other.

In the present study, WB and MAT (Table 3 and Table 4) had an intermediate agreement with the Toxotest kit (Gwet's AC_1= 0.458 and 0.557), similar to the report by Bărburaș *et al.* (2019). The closeness of Toxotest kit as an ELISA method with MAT and WB demonstrates a consistent pattern with other reports such as Dubey *et al.* (2005), Sroka *et al.* (2008), Zhu *et al*. (2012), Gu *et al.* (2015), and Galat *et al*. (2019). Therefore, the Toxotest kit can be considered to be applied together with or complementary to the WB and MAT. In contrast, the IDScreen, Toxo Ab, and Pastorex kits had a poor agreement with both MAT and WB (Gwet's AC_1= ≤0.2). These results were similar to that by Sroka *et al.* (2008) who also reported poor agreement between MAT and the Pastorex kit. However, this evidence differs from the report from Baso *et al.* (2020) who stated that the IDScreen kit had an almost perfect agreement with WB.

The good agreement between Toxo Ab and IDScreen is likely due to the similarity of the type of antigen used, namely the p30 recombinant protein that is coated on the microplate (Valinata *et al*., 2020). On the other hand, the Toxotest kit uses tachyzoite lysates from *Toxoplasma gondii* (Valinata *et al*., 2020), so it has a widely different range of test results from those of

Table 2. The agreement value among four Commercial ELISA Kits in establishing seropositive toxoplasmosis

Methods	Pastorex vs Toxotest			Pastorex vs ID Screen			Pastorex vs Toxo Ab		
	Coeff.	SE	95% C.I	Coeff.	SE	95% C.I	Coeff.	SE	95% C.I
Cohen's Kappa	-0.001	0.049	(-0.098, 0.097)	0.134	0.069	(-0.001, 0.27)	-0.041	0.051	(-0.142, 0.060)
Scott's Pi	-0.087	0.059	(-0.203, 0.029)	0.125	0.070	(-0.014, 0.264)	-0.144	0.061	(-0.263, -0.024)
Krippendorff's Alpha	-0.084	0.059	(-0.2, 0.032)	0.127	0.070	(-0.012, 0.266)	-0.141	0.061	(-0.26, -0.021)
Gwet's AC_1	0.136	0.052	(0.033, 0.239)	0.494	0.050	(0.396, 0.592)	0.511	0.049	(0.414, 0.607)
Brennan – Prediger	0.073	0.053	(-0.033, 0.178)	0.411	0.054	(0.305, 0.517)	0.395	0.054	(0.289, 0.502)
Percent Agreement	0.382	0.036	(0.311, 0.452)	0.608	0.036	(0.537, 0.678)	0.597	0.036	(0.526, 0.668)

Methods	Toxotest vs ID Screen			Toxotest vs Toxo Ab			ID Screen vs Toxo Ab		
	Coeff.	SE	95% C.I	Coeff.	SE	95% C.I	Coeff.	SE	95% C.I
Cohen's Kappa	0.105	0.042	(0.023, 0.187)	0.069	0.025	(0.019, 0.119)	0.039	0.062	(-0.082, 0.161)
Scott's Pi	-0.013	0.059	(-0.129, 0.103)	-0.206	0.057	(-0.318, -0.095)	-0.006	0.068	(-0.141, 0.129)
Krippendorff's Alpha	-0.010	0.059	(-0.127, 0.106)	-0.203	0.057	(-0.315, -0.091)	-0.003	0.068	(-0.138, 0.131)
Gwet's AC_1	0.176	0.053	(0.07, 0.28)	0.119	0.052	(0.016, 0.223)	0.623	0.045	(0.534, 0.713)
Brennan-Prediger	0.121	0.054	(0.014, 0.228)	0.032	0.053	(-0.072, 0.136)	0.524	0.051	(0.423, 0.625)
Percent Agreement	0.414	0.036	(0.343, 0.485)	0.355	0.035	(0.286, 0.424)	0.683	0.034	(0.615, 0.75)

Table 3. The agreement value between the four commercial ELISA kits with Immunoblotting in determining seropositive toxoplasmosis

	Immunoblotting vs Pastorex			Immunoblotting vs Toxotest			Immunoblotting vs ID Screen			Immunoblotting vs Toxo Ab		
	Coeff	SE	95% C.I	Coeff	SE	95% C.I	Coeff	SE	95% C.I	Coeff	SE	95% C.I
Cohen's Kappa	-0.039	0.056	(-0.151, 0.072)	0.211	0.102	(0.009, 0.414)	-0.027	0.057	(-0.142, 0.087)	0.022	0.012	(-0.002, 0.046)
Scott's Pi	-0.395	0.097	(-0.588, -0.202)	0.183	0.111	(-0.038, 0.405)	-0.353	0.099	(-0.549, -0.156)	-0.561	0.081	(-0.723, -0.400)
Krippendorff's Alpha	-0.387	0.097	(-0.580, -0.194)	0.188	0.111	(-0.033, 0.409)	-0.345	0.099	(-0.541, -0.149)	-0.553	0.081	(-0.714, -0.391)
Gwet's AC_1	-0.392	0.098	(-0.586, -0.197)	0.458	0.100	(0.260, 0.656)	-0.344	0.101	(-0.544, -0.144)	-0.496	0.102	(-0.698, -0.294)
Brennan-Prediger	-0.393	0.097	(-0.587, -0.200)	0.348	0.099	(0.151, 0.546)	-0.348	0.099	(-0.546, -0.151)	-0.528	0.090	(-0.707, -0.349)
Percent Agreement	0.303	0.049	(0.207, 0.400)	0.674	0.050	(0.575, 0.773)	0.326	0.050	(0.227, 0.425)	0.236	0.045	(0.147, 0.325)

Table 4. The agreement value between the four commercial ELISA kits with modified Agglutination test (MAT) in determining seropositive toxoplasmosis

	MAT vs Pastorex			MAT vs Toxotest			MAT vs ID Screen			MAT vs Toxo Ab		
	Coeff	SE	95% C.I	Coeff	SE	95% C.I	Coeff	SE	95% C.I	Coeff	SE	95% C.I
Cohen's Kappa	0.079	0.028	(0.023, 0.134)	0.288	0.093	(0.102, 0.473)	0.089	0.031	(0.026, 0.149)	0.013	0.008	(-0.002, 0.028)
Scott's Pi	-0.317	0.099	(-0.514, -0.119)	0.233	0.115	(0.005, 0.462)	-0.278	0.100	(-0.478, -0.078)	-0.673	0.076	(-0.824, -0.523)
Krippendorff's Alpha	-0.309	0.099	(-0.507, -0.112)	0.238	0.115	(0.009, 0.446)	-0.271	0.100	(-0.470, -0.071)	-0.664	0.076	(-0.814, -0.514)
Gwet's AC_1	-0.290	0.105	(-0.499, -0.082)	0.557	0.090	(0.377, 0.736)	-0.239	0.108	(-0.453, -0.026)	-0.653	0.084	(-0.820, -0.485)
Brennan-Prediger	-0.303	0.101	(-0.504, -0.103)	0.438	0.095	(0.249, 0.628)	-0.258	0.102	(-0.462, -0.055)	-0.663	0.079	(-0.821, -0.505)
Percent Agreement	0.348	0.051	(0.248, 0.449)	0.719	0.048	(0.624, 0.814)	0.371	0.051	(0.269 , 0.473)	0.169	0.039	(0.09, 0.247)

the previous two ELISA kits. The use of tachyzoite lysate as an antigen will result in the best ELISA, having high sensitivity and specificity, as reported by Abdelbaset *et al*. (2017). Therefore, it can be understood why the Toxotest kit detects more seropositive samples than the other kits and its test results are compatible with those of MAT and WB as all of them use whole tachyzoite and tachyzoite lysate as antigens.

As previously mentioned, eight disease investigation centers in Indonesia have been using four different serological test kits. This has lead to an informational bias regarding the national prevalence of toxoplasmosis. Some of the centers are even known to use two incompatible commercial kits in their tests. The recommended attempt to reduce informational bias regarding the prevalence of toxoplasmosis is to select two or more kits that have good agreement results. For example, if they want to use the IDScreen kit, then the commercial kit that has the best agreement is the Toxo Ab kit. Therefore, the eight disease investigation centers should choose one or both of the two kits. On the other hand, if they wish to carry out a more sensitive serological test for the detection of toxoplasmosis, it is advisable to use the Toxotest kit. Consequently, all eight disease investigation centers in Indonesia should use the same kit. If uniformity is not possible, then the recommended serological test is WB or MAT because they have an adequate agreement with the Toxotest kit.

CONCLUSION

IDScreen had a good agreement with Toxo Ab (Gwet's AC_1= 0.623) and a moderate agreement with Pastorex (Gwet's AC_1= 0.494-0.511). Toxotest had a low agreement with three commercial kits (Gwet's AC_1= <0.2) but had a moderate agreement with WB and MAT (Gwet's AC_1= 0.458-0.557). In general, the four commercial kits and the other two test methods can be separated into two groups based on the similarity of the diagnostic test results. The first group consists of Toxo Ab, IDScreen, and Pastorex kits while the other groups are the Toxotest kit, MAT and WB.

ACKNOWLEDGMENTS

Our gratitude to Mrs. Suyati Supardi who helped during the laboratory tests and cell culture for Toxoplasma propagation.

REFERENCES

Abdelbaset, E.A., H. Alhasan, D. Salman, M.H. Karram, M.A.E Rushdi, X. Xuenan, and M. Igarashi. 2017. Evaluation of recombinant antigens in combination and single formula for diagnosis of feline toxoplasmosis. **Exp. Parasitol.** 172:1-4.

Al-Adhami, B.H. and A.A. Gajadhar. 2014. A new multi-host species indirect ELISA using protein A/G conjugate for detection of anti-*Toxoplasma gondii* IgG antibodies with comparison to ELISA-IgG, agglutination assay and Western blot. **Vet. Parasitol.** 200:66-73.

Al-Adhami, B.H., M. Simard, A. Hernández-Ortiz, C. Boireau, and A.A. Gajadhar. 2016. Development and evaluation of a modified agglutination test for diagnosis of Toxoplasma infection using tachyzoites cultivated in cell culture. **Food Waterborne Parasitol.** 2:5-21.

Altman, D.G. 1991. **Practical Statistics for Medical Research**. Chapman and Hall, London.

Banjarbaru DIC. 2019. **Map of Animal Diseases in Regional V (Borneo).** Banjarbaru Diseases Investigation Center, Directorate of Animal Health, General Director of Livestock and Animal Health, Ministry of Agriculture of Indonesia.

Bărburaș, D., A. Györke, R. Blaga, R. Bărburaș, Z. Kalmár, S. Vișan, V. Mircean, A. Blaizot, and V. Cozma. 2019. *Toxoplasma gondii* in water buffaloes (*Bubalus bubalis*) from Romania: what is the importance for public health?. **Parasitol. Res.** 118:2695–2703. https://doi.org/10.1007/s00436-019-06396-6.

Basso, W., E. Sollberger, G. Schares, S. Küker, F. Ardüser, G. Moore-Jones, and P. Zanolari. 2020. *Toxoplasma gondii* and *Neospora caninum* infections in South American camelids in Switzerland and assessment of serological tests for diagnosis. **Parasit. Vectors.** 13:256. Doi:10.1186/s13071-020-04128-9.

Blaga, R., D. Aubert, A. Thébault, C. Perret, R. Geers, M. Thomas, A. Alliot, V. Djokic, N. Ortis, L. Halos, B. Durand, A. Mercier, I. Villena, and P. Boireau. 2019. *Toxoplasma gondii* in beef consumed in France: regional variation in seroprevalence and parasite isolation. **Parasite.** 26:77. Doi:10.1051/parasite/ 2019076.

Bukittinggi DIC. 2019. **Map of Animal Diseases in Regional II (West Sumatra, Riau, Riau Island, Jambi).** Bukittinggi Diseases Investigation Center, Directorate of Animal Health, General Director of Livestock and Animal Health, Ministry of Agriculture of Indonesia.

Capobiango, J.D., T.C. Monica, F.P. Ferreira, R. Mitsuka-Breganó, I.T. Navarro, and J.L. Garcia. 2016. Evaluation of the Western blotting method for the diagnosis of congenital toxoplasmosis. **J. Pediatr. (Rio J).** 92(6):616-623.

Dubey, J.P., G. Desmonts, C. McDonald, and K.W. Walls. 1985. Serologic evaluation of cattle inoculated with *Toxoplasma gondii*: comparison of Sabin-Feldman dye test and other agglutination tests. **Am. J. Vet. Res.** 46(5):1085-1088.

Dubey, J.P. and G. Desmonts. 1987. Serological responses of equids fed with *Toxoplasma gondii* oocysts. **Equine Vet. J.** 48:1239-1243.

Dubey, J.P., P.A. Fair, G.D. Bossart, D. Hill, R. Fayer, C. Sreekumar, O.C.H. Kwok, and P. Thulliez. 2005. A Comparison of several serologic test to detect antibodies to *Toxoplasma gondii* in naturally exposed bottlenose dolphins (*Tursiops truncatus*). **J. Parasitol.** 91(5):1074–1081.

Dubey, J.P., I. Hotea, T.R. Olariu, J.L. Jones, and G. Dărăbuș. 2014. Epidemiological review of toxoplasmosis in humans and animals in Romania. **Parasitology.** 141:311-325.

Elfahal, A.M., A.M. Elhassan, M.O. Hussien, K.A. Enan, A.B. Musa, and A.M. El Hussein. 2013. Seroprevalence of *Toxoplasma gondii* in dairy cattle with reproductive problems in Sudan. **ISRN Vet. Sci.** 2013:1-4.

Fajardo, H.V., S. D'ávila, R.R. Bastos, C.D. Cyrino, M.L. Detoni, J.L. Garcia, L.B. Neves, J.L. Nicolau, and M.R.R. Amendoeira. 2013. Seroprevalence and risk factors of toxoplasmosis in cattle from extensive and semi-intensive rearing systems at Zona da Mata, Minas Gerais state, Southern Brazil. **Parasit. Vectors.** 6:191. doi:10.1186/1756-3305-6-191.

Galat, M., K. Must, K. Rissanen, and P. Jokelainen. 2019. Comparison of a commercial modified direct agglutination test and a commercial enzyme-linked immunosorbent assay for screening for antibodies against *Toxoplasma gondii* in naturally exposed domestic cats. **Parasitol. Res.** 118:2437-2441.

Gamble, H.R, J.P. Dubey, and D.N. Lambillotte. 2005. Comparison of a commercial ELISA with the modified agglutination test for detection of Toxoplasma infection in the domestic pig. **Vet. Parasitol.** 128:177-181.

Garcia, J.L., S.M. Gennari, I.T. Navarro, R.Z. Machado, S.A. Headley, O. Vidotto, J.S.G. Junior, F.M. Bugni, and M. Igarashi. 2008. Evaluation of IFA, MAT, ELISAs and immunoblotting for the

detection of anti-*Toxoplasma gondii* antibodies in paired serum and aqueous humour samples from experimentally infected pigs. **Res. Vet. Sci.** 84:237-242.

Gu, Y., Z. Wang, Y. Cai, X. Li, F. Wei, L. Shang, J. Li, and Q. Liu. 2015. A comparative study of *Toxoplasma gondii* seroprevalence in mink using a modified agglutination test, a Western blot, and enzyme-linked immunosorbent assays. **J. Vet. Diagn. Invest.** 27(5):616-620.

Gwet, K.L. 2016. Testing the difference of correlated agreement coefficients for statistical significance. **Educ. Psychol. Meas.** 76(4):609-637.

Kurniawan, A., I.P. Sari, N. Harminarti, L. Edwar, and M. Susiyanti. 2020. *Toxoplasma gondii* SAG2 type III in an atypical presentation of ocular toxoplasmosis in Indonesia. **Int. J. Infect. Dis.** 96:440-444.

Molan, A., K. Nosaka, M. Hunter, and W. Wang. 2019. Global status of *Toxoplasma gondii* infection: Systematic review and prevalence snapshots. **Trop. Biomed.** 36(4):898-925.

Montoya, J.G. 2002. Laboratory Diagnosis of Toxoplasma gondii Infection and Toxoplasmosis. **J. Infect. Dis.** 185:73-82.

Sroka, J., T. Cencek, I. Ziomko, J. Karamon, and J. Zwoliński. 2008. Preliminary assesment of ELISA, MAT, and LAT for detecting *Toxoplasma gondii* antibodies in pigs. **Bull. Vet. Inst. Pulawy**. 52:545-549.

Steinparzer, R., K. Reisp, B. Grünberger, J. Köfer, F. Schmoll, and T. Sattler. 2014. Comparison of Different Commercial Serological Tests for the Detection of *Toxoplasma gondii* Antibodies in Serum of Naturally Exposed Pigs. **Zoonoses Public Health.** 62(2):119-124.

Tagwireyi, W.M., E. Eter, and L. Neves. 2019, Seroprevalence and associated risk factors of *Toxoplasma gondii* infecton in domestic animals in southeastern South Africa. **Onderstepoort J. Vet. Res.** 86(1): a1688. Doi:10.4102/ojvr.v86i1.1688.

Terazawa, A., R. Muljono, L. Susanto, S.S. Margono, and E. Konishi. 2003. High Toxoplasma antibody prevalence among inhabitants in Jakarta, Indonesia. **Jpn. J. Infect. Dis.** 56:107-109.

Valinata, S., Sulinawati, and D.T. Subekti. 2020. Evaluation of Assay performance and inter reliability agreement betwen Toxoplasma modified agglutination test and several commercially serological assay kits. **J. Veteriner.** 21(2):278-291.

Wates DIC. 2018. **Map of Animal Diseases in Regional IV (East Jawa, Central Jawa and Jogyakarta).** Wates Diseases Investigation Center, Directorate of Animal Health, General Director of Livestock and Animal Health, Ministry of Agriculture of Indonesia.

Wulandari, R., J.F. Suwandi, H. Mutiara, Sulinawati, and R. Hanriko. 2019. Seroprevalensi *Toxoplasma gondii* in Cattle in Bandar Lampung. **J. Agromedicine.** 6(1):1-5.

Yu, J., Z. Xia, Q. Liu, J. Liu, J. Ding, and W. Zhang. 2007. Seroepidemiology of *Neospora caninum* and *Toxoplasma gondii* in cattle and water buffaloes (*Bubalus bubalis*) in the People's Republic of China. **Vet. Parasitol.** 143(1):79-85.

Zhu, C.H., L.L. Cui, and L.S. Zhang. 2012. Comparison of a commercial ELISA with the modified agglutination test for detection of *Toxoplasma gondii* antibodies in sera of naturally infected dogs and cats. **Iran. J. Parasitol.** 7(3):89-95.