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

Didik T Subekti1*, Sisca Valinata2, and Sulinawati Fong2
1Indonesian Research Center for Veterinary Sciences, Bogor, Indonesia
2Lampung 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 AC² = 0.623) and a moderate agreement with Pastorex (Gwet's AC² = 0.494-0.511). Toxotest had a low agreement with three commercial kits (Gwet's AC² = <0.2) but had a moderate agreement with western blotting (WB) and modified agglutination test (MAT) (Gwet's AC² = 0.458-0.557).

Key words: cattle, ELISA, seroprevalence, toxoplasmosis

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 et al., 2003; Capobianco 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 (El Fahal 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. West Sumatra DIC and Lampung DIC, 2018). Toxoplasmosis seroprevalence in cattle in West Sumatra was reported by DIC, 2019. Meanwhile, a 47.75% serorevalence of toxoplasmosis in cattle in West Sumatra was reported by DIC, 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, 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.
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 Protein® 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 Protein (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
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 AC₁, 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 IDSscreen 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 IDSscreen 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 IDSscreen 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 IDSscreen 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](image-url)
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

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

<table>
<thead>
<tr>
<th>Sample Origin</th>
<th>Seropositive (%)</th>
<th>Seronegative (%)</th>
<th>Suspect/Doubtful (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pastor ex</td>
<td>Toxotest ID Screen</td>
<td>Toxo Ab Pastor ex</td>
</tr>
<tr>
<td>Liwa, West Lampung</td>
<td>20 100 20 0 80 0 80 100</td>
<td>0 0 0 0 0 0 0 0</td>
<td></td>
</tr>
<tr>
<td>Malangkari, South Lampung</td>
<td>0 100 60 0 40 0 40 100</td>
<td>0 0 0 0 0 0 0 0</td>
<td></td>
</tr>
<tr>
<td>Pesawaran</td>
<td>80 100 30 0 70 0 70 100</td>
<td>0 0 0 0 0 0 0 0</td>
<td></td>
</tr>
<tr>
<td>Candipuro, South Lampung</td>
<td>22.5 100 50 32.5 77.5 0 50 67.5 0 0 0 0 0 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abung Timur, North Lampung</td>
<td>4 100 4 4 96 0 96 96 0 0 0 0 0 0 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sungkai Utara, North Lampung</td>
<td>20 0 0 0 80 100 100 100 0 0 0 0 0 0 0 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rama Puja, East Lampung</td>
<td>0 33.3 33.3 0 100 66.67 66.67 100 100 0 0 0 0 0 0 0 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Manna, South Bengkulu</td>
<td>54.6 13.6 19.7 4.6 45.5 57.58 72.7 95.5 0 28.8 7.6 0 0 0 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Musi Banyuaas, South Sumatra</td>
<td>50 25 25 0 50 50 75 100 0 25 0 0 0 0 0 0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

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The Toxotest kit uses tachyzoite lysates and is coated on the microplate (Valinata et al., 2019). 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 Bărbaș 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

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

<table>
<thead>
<tr>
<th>Methods</th>
<th>Pastorex vs Toxotest</th>
<th>Pastorex vs IDScreen</th>
<th>Pastorex vs Toxo Ab</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coeff.</td>
<td>SE</td>
<td>95% C.I</td>
</tr>
<tr>
<td>Cohen’s Kappa</td>
<td>-0.001</td>
<td>0.049</td>
<td>(-0.009, 0.097)</td>
</tr>
<tr>
<td>Scott’s Pi</td>
<td>-0.087</td>
<td>0.059</td>
<td>(-0.203, 0.029)</td>
</tr>
<tr>
<td>Krippendorf’s Alpha</td>
<td>-0.084</td>
<td>0.059</td>
<td>(-0.2, 0.032)</td>
</tr>
<tr>
<td>Gwet’s AC₁</td>
<td>0.136</td>
<td>0.052</td>
<td>(0.033, 0.239)</td>
</tr>
<tr>
<td>Brennan – Prediger</td>
<td>0.073</td>
<td>0.053</td>
<td>(-0.033, 0.178)</td>
</tr>
<tr>
<td>Percent Agreement</td>
<td>0.382</td>
<td>0.036</td>
<td>(0.311, 0.452)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Immunoblotting vs Pastorex</th>
<th>Immunoblotting vs Toxotest</th>
<th>Immunoblotting vs IDScreen</th>
<th>Immunoblotting vs Toxo Ab</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coeff.</td>
<td>SE</td>
<td>95% C.I</td>
</tr>
<tr>
<td>Cohen’s Kappa</td>
<td>-0.039</td>
<td>0.056</td>
<td>(-0.151, 0.072)</td>
</tr>
<tr>
<td>Scott’s Pi</td>
<td>-0.395</td>
<td>0.097</td>
<td>(-0.588, 0.020)</td>
</tr>
<tr>
<td>Krippendorf’s Alpha</td>
<td>-0.387</td>
<td>0.097</td>
<td>(-0.580, 0.184)</td>
</tr>
<tr>
<td>Gwet’s AC₁</td>
<td>-0.392</td>
<td>0.098</td>
<td>(-0.586, 0.017)</td>
</tr>
<tr>
<td>Brennan-Prediger</td>
<td>-0.393</td>
<td>0.097</td>
<td>(-0.587, 0.200)</td>
</tr>
<tr>
<td>Percent Agreement</td>
<td>0.303</td>
<td>0.049</td>
<td>(0.207, 0.400)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>MAT vs Pastorex</th>
<th>MAT vs Toxotest</th>
<th>MAT vs IDScreen</th>
<th>MAT vs Toxo Ab</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coeff.</td>
<td>SE</td>
<td>95% C.I</td>
<td>Coeff.</td>
</tr>
<tr>
<td>Cohen’s Kappa</td>
<td>0.079</td>
<td>0.028</td>
<td>(0.023, 0.134)</td>
</tr>
<tr>
<td>Scott’s Pi</td>
<td>-0.317</td>
<td>0.099</td>
<td>(-0.514, -0.119)</td>
</tr>
<tr>
<td>Krippendorf’s Alpha</td>
<td>-0.309</td>
<td>0.099</td>
<td>(-0.507, -0.112)</td>
</tr>
<tr>
<td>Gwet’s AC₁</td>
<td>-0.290</td>
<td>0.105</td>
<td>(-0.493, -0.082)</td>
</tr>
<tr>
<td>Brennan-Prediger</td>
<td>-0.303</td>
<td>0.101</td>
<td>(-0.504, -0.033)</td>
</tr>
<tr>
<td>Percent Agreement</td>
<td>0.348</td>
<td>0.051</td>
<td>(0.248, 0.449)</td>
</tr>
</tbody>
</table>
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 led 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 achieved, it is advisable to use the Toxotest kit for toxoplasmosis. Some of the centers are even known to use the Toxo Ab kit.

CONCLUSION

IDScreen had a good agreement with Toxo Ab (Gwet’s AC^I = 0.623) and a moderate agreement with Pastorex (Gwet’s AC^I = 0.494-0.511). Toxotest had a low agreement with three commercial kits (Gwet’s AC^I = <0.2) but had a moderate agreement with WB and MAT (Gwet’s AC^I = 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.

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