Clusters of Toxoplasmosis in Gyodong-Myeon and Samsan-Myeon, Ganghwa-Gun, Korea

INTRODUCTION

Toxoplasma gondii, an apicomplexan zoonotic protozoan, is one of the most widespread parasites worldwide. Warm blooded animals are its hosts, especially felids. Toxoplasmosis is acquired by the infection with T. gondii through undercooked meat, contaminated water, or mother to her fetus. Congenital toxoplasmosis may cause critical damages to the fetus [1-5]. For adult humans, most of their symptoms are light or asymptomatic. However, the infections can be exacerbated into chronic conditions and can cause a serious diseases in immune compromised patients [6-9]. Rapid diagnostic test (RDT) is a tool for diagnosis of toxoplasmosis. Seroprevalence of toxoplasmosis in certain areas is surging recently [10-13]. Previous studies have shown that the overall proportion, gender and age distribution of Gyodong-Myeon and Samsan-Myeon [11,12]. In addition to this, if clusters with high risk are specified by comparing them to each region, efficient monitoring and health policies can be established accordingly.

Spatial scan statistic and Kulldorff’s SaTScan Software were used to detect the clusters. This study analyzed clusters using RDT for the detection of anti-T. gondii antibodies with the sera of residents in Gyodong-Myeon, Samsan-Myeon both in Ganghwa-gun, Incheon, Korea for 4 years from 2010 to 2013.

MATERIALS AND METHODS

Ethics statement

This study was performed under the regulation of the IRB Committee of The Catholic University of Korea (No. MIRB-20200710-003). This research adhered to the tenets of the Declaration of Helsinki.

Rapid diagnostic test (RDT)

RDT data with address of subjects from Gyodong-Myeon and Samsan-Myeon was obtained from preceding studies, and the number of patients in each region is summarized in the Table 1 [11,12]. IgG/IgM RDT mounted with recombinant fragment of major surface antigen (SAG1A), GST-linker-SAG1A, were applied to the sera mentioned above [14]. Briefly, 10 µl of serum was applied to the RDT sample hole and eluted with RDT buffer, i.e., 0.1% casein and 1% Tween 20 in 0.1 M
Tris-HCl buffer (pH 8.0) a few seconds later. Reacting bands were read by 3 investigators after 15-20 min, and its density was determined arbitrarily as 0, +, ++, and ++++. Definite reactivity was determined as ++ (mid), while weaker and stronger reactivity compared to it was determined as + (weak) and ++++ (strong). The final results were agreed upon by the 3 investigators.

Spatial scan statistic

Spatial scan statistics continually change the size and location of predefined shaped windows and calculate the relative risk of risk within and outside the window for the region of interest, identifying clusters with relatively high risk compared to other regions [15]. Satscan increases the size gradually around each local unit, making circular windows that overlap each other hierarchical. Calculate the likelihood ratio for all windows and obtain the maximum likelihood ratio and use it as a test statistic. The Monte Carlo test is performed under the null hypothesis that the cluster does not exist (RR = 1, the risk is equal in all areas). First, we calculate the test statistic from the actual data to create a random set of data under the null hypothesis. The test statistics are calculated from each of these random repetitive datasets. Then, if it ranks in the upper alpha % by aligning the preceding test statistics, the null hypothesis is rejected under a significant level of alpha % and the P-value is calculated [16-19].

Shown in Table 1, a valuable cluster analysis of IgM-RDT cannot be obtained as the incidence of positive patients in IgM-RDT is significantly low, thus the analysis was only performed on IgG-RDT. Since the results of IgG-RDT are discrete at 0, +, ++, and ++++, and the proportion of positive patients is near or above 20%, divided the results of IgG-RDT into positive and negative, and Bernoulli model used at 5% of significance level. The base point of each Ri shall be the latitude and longitude coordinates of the village hall (or community center) if there is no Ri office. If there are several halls, the coordinates of the midpoint were used. The window size is set to 25%, 15% per annum for the data in Gyodong-myeon and Samsan-myeon. In most cases, the upper cluster region and the relative risk were the same when the maximum allowed window was 15% and 25%, while the P-value was lower when it was 15%. Therefore, only clusters with a maximum allowed window size of 15% were used for the result analysis. In Table 2 and Table 3, relative risk indicates how much the risk of the cluster and surrounding areas varies. Log likelihood ratio is an indicator of how many times positive patients will occur in the cluster than a non-cluster. The greater the P-value, the less sig-

Table 1. The number of sera, IgM-RDT positive number, and IgG-RDT positive number of each regions in Gyodong-myeon and Samsan-myeon from 2010 to 2013

| Year | No. of sera | IgM-RDT (%) | IgG-RDT (%) |
|------|-------------|-------------|-------------|
| Gyodong-myeon | | | |
| 2010 | 921 | 3 (0.3) | 191 (20.7) |
| 2011 | 999 | 1 (0.1) | 243 (24.3) |
| 2012 | 929 | 7 (0.8) | 251 (27.0) |
| 2013 | 848 | 13 (1.5) | 226 (26.7) |
| Samsan-myeon | | | |
| 2010 | 313 | 1 (0.3) | 84 (26.8) |
| 2011 | 342 | 1 (0.3) | 107 (31.3) |
| 2012 | 379 | 29 (7.7) | 137 (36.1) |
| 2013 | 361 | 5 (1.4) | 183 (50.7) |

Table 2. Clusters detected from Gyodong-myeon by year and 4-year average

| Year | Cluster no. | Location | Case | Relative risk | Log likelihood ratio | P-value |
|------|-------------|----------|------|---------------|---------------------|---------|
| Average | 1 | Jiseok | 25 | 1.734 | 4.15 | 0.032 |
| | 2 | Muhak | 15 | 1.175 | 0.236 | 0.995 |
| | 3 | Yanggap | 20 | 1.15 | 0.23 | 0.997 |
| 2010 | 1 | Jiseok | 25 | 1.951 | 5.533 | 0.01 |
| | 2 | Bongso | 20 | 1.247 | 0.527 | 0.929 |
| | 3 | Gogu | 25 | 1.17 | 0.33 | 0.977 |
| 2011 | 1 | Muhak | 41 | 1.473 | 3.206 | 0.103 |
| | 2 | Bongso | 21 | 1.43 | 1.526 | 0.489 |
| | 3 | Yanggap | 23 | 1.288 | 0.844 | 0.809 |
| 2012 | 1 | Jiseok | 29 | 1.654 | 4.174 | 0.035 |
| | 2 | Sangyong Bongso | 22 | 1.256 | 0.69 | 0.877 |
| | 3 | Yanggap | 27 | 1.182 | 0.448 | 0.961 |
| 2013 | 1 | Jiseok | 24 | 1.913 | 5.733 | 0.006 |
| | 2 | Seohan | 20 | 1.259 | 0.638 | 0.919 |
significant the cluster is. Conversely, the smaller the $P$-value, the more certain the area is a cluster. The R program was used to visualize clusters on the map.

**RESULTS**

Using Satscan, the average and annual clusters of each region of Gyodong-myeon and Samsan-myeon were analyzed and marked on the map according to the relative risk of each cluster. A cluster with a $P$-value less than 0.05 is significant, but all clusters are plotted for visual comparison. If the cluster contains multiple regions, all are marked.

**Gyodong-myeon**

In the 4-year average of Gyodong-myeon, Jiseok-ri is the most likely cluster and is significant at a significant level of 5%, with Log likelihood ratio $= 4.150$, Relative risk $= 1.734$, and $P$-value $= 0.032$ ($< 0.05$), indicating the risk in Jiseok-ri was 1.734 times higher than other area (Table 2). Muhak-ri is the secondary and Yanggap-ri is the third cluster (both of $P$-value $> 0.05$). In 2010, Jiseok-ri was also the most likely cluster with Log likelihood ratio $= 5.533$, Relative risk $= 1.951$, $P$-value $= 0.01$ ($< 0.05$), indicating the risk in Jiseok-ri was 1.951 times higher than other area. Bongso-ri and Gogu-ri are second and third, respectively. In 2011, Muhak-ri had Log likelihood ratio $= 3.206$, Relative risk $= 1.473$, $P$-value $= 0.103$, which makes it hard to say that the first cluster because $P$-value is greater than 0.05. Bongso-ri is the second cluster and Yanggap-ri is the third. In 2012, Log likelihood ratio $= 4.174$, Relative risk $= 1.654$, $P$-value $= 0.035$ ($< 0.05$) in Jiseok-ri, the first cluster. The second is Sangyong-ri, the third is Yanggap-ri and both $P$-value exceeded 0.05. In 2013, Log likelihood ratio $= 5.733$, Relative risk $= 1.913$, $P$-value $= 0.006$ ($< 0.05$) Jiseok-ri is the most likely cluster and Seohan-ri is the second. Except for 2011, which falls short of the $P$-value standard, Jiseok-ri consistently appears as a cluster in Gyodong-myeon (Fig. 1).

**Table 3.** Clusters detected from Samsan-myeon by year and 4-year average

| Year | Cluster no. | Location | Case | Relative risk | Log likelihood ratio | $P$-value |
|------|-------------|----------|------|---------------|----------------------|----------|
| Average | 1 | Ha | 24 | 1.711 | 3.945 | 0.002 |
| | 2 | Seogeom | 15 | 1.17 | 0.235 | 0.609 |
| | 3 | Sang | 8 | 1.178 | 0.146 | 0.748 |
| 2010 | 1 | Ha | 42 | 1.886 | 3.833 | 0.015 |
| | 2 | Sang | 21 | 1.07 | 0.017 | 0.856 |
| 2011 | 1 | Seogeom | 15 | 1.165 | 0.214 | 0.685 |
| | 2 | Sang | 8 | 1.071 | 0.025 | 0.89 |
| 2012 | 1 | Ha | 29 | 1.993 | 8.469 | <0.001 |
| | 2 | Seogeom | 23 | 1.28 | 0.998 | 0.322 |
| | 3 | Sang | 3 | 1.19 | 0.068 | 0.885 |
| 2013 | 1 | Ha | 20 | 1.433 | 1.63 | 0.102 |
| | 2 | Sang | 14 | 1.379 | 0.961 | 0.341 |
| | 3 | Seogeom | 14 | 1.242 | 0.441 | 0.584 |

**Fig. 1.** Toxoplasmosis clusters in Gyodong-myeon by year. The higher the relative risk, the red the darker.
Samsan-myeon

In the 4-year average of Samsan-myeon, Ha-ri is significant as the most likely cluster with LLR = 3.955, RR = 1.71 and P-value = 0.002 (<0.05), indicating the risk in Ha-ri was 1.71 times higher than other area (Table 3). The second cluster is Seogoeom-ri and the third is Sang-ri. In 2010, the most likely cluster is Ha-ri for LLR = 3.833, Relative risk = 1.886, and P-value = 0.015 (<0.05), indicating the risk in Ha-ri was 1.886 times higher than other area, and Sang-ri is the second cluster. In 2011, the result is not taken into account because the P-value of the primary cluster, Seogoeom-ri, is 0.685, well above 0.05. Sang-ri is the second in 2011. In 2012, Ha-ri is the most likely cluster with LLR = 8.469, RR = 1.993, and P-value is less than 0.001, indicating the risk in Ha-ri in 2012 was 1.993 times higher than other area. Seogoeom-ri is the second, Sang-ri is the third. In 2013, Ha-ri is primary cluster but P-value fell short of the standard at 0.102. Sang-ri and Seogoeom-ri are second and third, respectively. In the results of the Satscan analysis in Samsan-myeon, the P-value of all the second and third clusters is greater than 0.05, that is, those are not statistically insignificant. Except for 2011 and 2013, the cluster in Samsan-myeon is Ha-ri (Fig. 2).

**DISCUSSION**

This study provided clusters with a high risk of toxoplasmosis infection in Gyodong-myeon and Samsan-myeon, Ganghwa-gun, Incheon, Korea through spatial scan analysis from 2010 to 2013. At the maximum allowed window size of 15%, it was generally shown as Jiseok-ri is the most likely cluster in Gyodong-myeon for 4 years. In Samsan-myeon, the most likely cluster is Ha-ri.

Gyodong-myeon is the northernmost island of the Military Demarcation Line. Most of the islands are surrounded by iron fences, most residents are engaged in agriculture rather than fishing. There is a wharf on the east side of the island, and recently it is connected to Ganghwa-do island by yeonpyeong bridge. Gyodong-island is a region where toxoplastic lymphadenitis had a group outbreak in residents who ingested pork raised at home in 1997 [7]. Micro cluster in the island is located in the village in the northwest, and toxoplastic environment by cats is maintained until now. It is estimated that the better conditions are presented for the spread of infectious sources due to the recent introduction of organic farming methods in agricultural areas.

Samsan-myeon is the administrative district of Seokmodo-do, where the cluster is identified with a similar pattern to Gyodong-myeon above. Micro-clusters are formed in the northern agricultural area of the island rather than in the area near the wharf in the southwest and recently connected to the main island of Ganghwa-do by the bridge.

Therefore, cluster analysis in relatively narrow islands in this
study can estimate whether the source of \( T. gondii \) is oocyst mediated by cats, or tissue-cyst mediated by food and mainly pork, and can exclude \( T. gondii \) infection environments from farming structures. The findings can be used to monitor and prevent toxoplasmosis infections occurring in vulnerable areas.

**CONFLICT OF INTEREST**

The authors declare no conflict of interest related to this study.

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