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Validation of immunoperoxidase technique for the diagnosis of toxoplasmosis in Nigerian wild rats

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Abstract

Toxoplasmosis is a highly prevalent parasitic zoonosis caused by *Toxoplasma gondii*. It is rated the 4th most important food-borne disease by the Food and Agriculture Organization (FAO). Several diagnostic tests have been used in the detection of toxoplasmosis with varied level of reliability. Among the several diagnostic tests, polymerase chain reaction (PCR) is regarded as the most sensitive. Immunoperoxidase technique has also been used in the diagnosis of toxoplasmosis. This, however, has not been validated in the detection of *Toxoplasma gondii* in rats. We compared the results of the use of immunoperoxidase technique in the detection of *Toxoplasma gondii* in wild rats to ascertain the level of agreement with the use of PCR. Results of 75 samples previously tested by PCR for which immunoperoxidase technique was also used to test the presence of *Toxoplasma gondii* were computed for this study. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated. The use of immunoperoxidase technique in the diagnosis of toxoplasmosis was 84.6% sensitive and 50% specific. The ability of immunoperoxidase technique to detect a positive test for toxoplasmosis was 91.7%. With the high sensitivity and PPV, we conclude that immunoperoxidase technique can be used as a screening tool for *Toxoplasma gondii* with reliable results.

Keywords: Immunoperoxidase technique, Polymerase chain reaction, Sensitivity, Specificity, *Toxoplasma gondii*

Introduction

Toxoplasmosis is one of the leading parasitic zoonoses worldwide caused by *Toxoplasma gondii*, a highly infectious apicomplexan parasite (Dubey *et al.,* 2021). The disease is rated 4th in the FAO list of food-borne diseases (Robertson *et al.,* 2013), and it is estimated that approximately 30% of the global human population is exposed to the disease (Dubey *et al.,* 2021). However, research on toxoplasmosis is yet to receive the needed attention as it has remained grossly underfunded (Hemphil *et al*., 2017). Several

diagnostic tests have been used over time for the detection of toxoplasmosis. Among the commonly used diagnostic procedures for the detection of *Toxoplasma gondii* are the latex agglutination test, Sabin-Feldman dye test, modified agglutination test, haemagglutination test, indirect fluorescent antibody test, and enzyme-linked immunosorbent assay, most of which are serological procedures (Hill *et al.,* 2005). With more recent advances in technology, other diagnostic procedures like western blotting and polymerase chain reaction (PCR) have been validated and utilized for the diagnosis of toxoplasmosis (Liu *et al.,* 2015). Molecular techniques are also regarded as the most sensitive procedures compared to the previous commonly used techniques (Lin *et al.,* 2000). Immunoperoxidase technique has also been used to detect *Toxoplasma gondii* in infected animals (Park *et al.,* 2007; Lopes *et al.,* 2011; Ode *et al.,* 2022a), but yet to be validated for use in rats as far as available literature is concerned. This paper aims to ascertain the validation of immunohistochemical detection of *T. gondii* as compared to the use of PCR for the detection of *T. gondii* in Nigerian wild rats.

Materials and Methods

Study area

This study was carried out in three states of North Central Nigeria, comprising Benue, Nasarawa, and Plateau states. Benue State is located between the latitudes 6°25'N and 8°8'N and longitude 7°47'E and 10°E, in the Tropical Guinea Savanna. It has an annual rainfall of 1000mm with a minimum temperature range of 27℃ to 28℃ and a maximum of 300℃ to 34℃ (Meteorological Department, Nigeria Air Force Base, Makurdi; unpublished data). Nasarawa State lies between the latitudes 7°45' and 9°25' of the equator and 9°37'E of the Greenwich Meridian. It has an annual rainfall of 1200 to 2000mm, a temperature of 22.5 - 27.5℃, and a relative humidity of 55 – 95% (Binbol *et al.,* 2020).

The climate of Plateau is tropical but cooler than the surrounding lowlands. Its average temperatures range from 15.5℃ to 18.5℃ in the coolest months to 27.5℃ to 30.5℃ during the hottest months. Rainfall ranges from 2,000 mm per year in the southwest to 1,500 mm or less in the drier northeast, with an average rainfall of 1,411 mm per year (Binbol *et al.,* 2020).

Study population

The wild rats used for this study were *Zyzomys pedunculatus*. Tissue samples from seventy-five (75) rats, which were previously screened for *T. gondii* using PCR (Ode *et al.,* 2022a) and whose pathology and immunohistochemical detection were already described (Ode *et al.,* 2022b), were utilized for this study.

Study design

Seventy-five samples, among which comprise both those reported positive and those negative for *T. gondii* by PCR were selected. For the selected samples, their corresponding immunoperoxidase technique results were obtained. Those that were positive by PCR and likewise positive by immunoperoxidase technique were recorded as the true positives. While those negative by PCR but positive by immunoperoxidase technique were recorded as false positives. Samples that were negative by PCR and likewise negative by immunoperoxidase technique were recorded as true negatives, while those positive by PCR but were negative by immunoperoxidase technique were recorded as false negatives.

Statistical analysis

The data obtained were subjected to statistics for the Altman and Bland determination of sensitivity, specificity, positive predictive value, and negative predictive value with slight modification as described by Adamu *et al.* (2020). The following equations were used to calculate the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV).

$$Sensitivity = \frac{True\ Positives}{(True\ Positives + False\ negatives)} X\ 100$$

$$Specificity = \frac{True\ Negatives}{(True\ Negatives + False\ negatives)} X\ 100$$

$$Negative\ Predictive\ Value\ (PVneg) = \frac{True\ Negatives}{(True\ Negatives + False\ negatives)} X\ 100$$

$$Positive\ Predictive\ Value\ (PVP) = \frac{True\ Positives}{(True\ Positives + False\ positives)} X\ 100$$

Results and Discussion

Table 1 shows the frequencies and percentages of test results by PCR in comparison with the immunoperoxidase technique. The use of immunoperoxidase technique in comparison with PCR in the detection of *T. gondii* in Nigerian wild rats revealed a sensitivity of 84.6%, specificity of 50%, PPV of 91.7% and NPV of 33.3%.

Table 1. Frequencies and percentages (in parentheses) of test results by polymerase chain reaction (PCR) in comparison with the immunoperoxidase technique in the detection of *Toxoplasma gondii* in wild rats captured in Nigeria

	Numbers positive by PCR	Numbers negative by PCR	Total
Numbers positive by immunoperoxidase technique	55 (91.7)	10 (66.7)	65 (86.6)
Numbers negative by immunoperoxidase technique	5 (8.3)	5 (33.3)	10 (13.3)
Total	60 (100)	15 (100)	10 (100)

The sensitivity and specificity of a diagnostic are used to ascertain how good a test is at predicting a disease condition. Both PCR and immunoperoxidase technique are well-recognized techniques used in the diagnosis of several disease conditions, including infectious diseases (Ode *et al.,* 2022a; Ode *et al.,* 2022b). Due to the sensitivity of PCR in detecting the presence of a pathogen, even in very little concentration, from the several cycles of amplification of the nucleic acids of the pathogen, it is regarded as a gold standard for the detection/diagnosis of several infectious diseases (Liu *et al.,* 2015). We tested this observation by comparing the results of the detection of *Toxoplasma gondii* in tissues of Nigerian wild rats using PCR with immunoperoxidase technique to check the level of concordance and estimate the rate of sensitivity and specificity of the use of immunoperoxidase technique in the detection of *Toxoplasma gondii* in rats. The sensitivity of immunoperoxidase technique in this report was 84.6%. This was higher than Breslin *et al. (*2000) in the use of immunoperoxidase technique to diagnose Turkey Coronavirus, but lower than the 91% sensitivity reported by Belaud-Rotureau *et al.* (2002) in the diagnosis of mantle cell lymphomas.

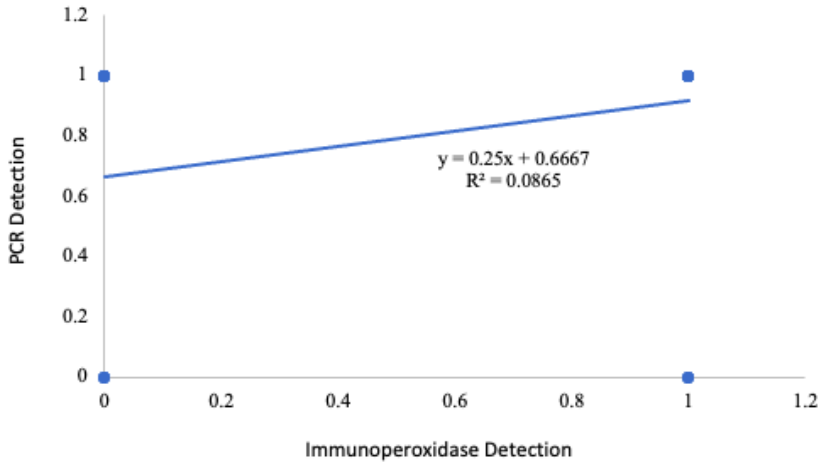

Figure 1: Correlation between polymerase chain reaction (PCR) and immunoperoxidase technique detection of Toxoplasma gondii in wild rats captured in Nigeria

We report a specificity of 50% in the use of immunoperoxidase technique for the detection of *Toxoplasma gondii* in rats. This is less than the 96% specificity of immunoperoxidase technique reported by Breslin *et al.* (2000). In validation of diagnostic techniques, however, higher sensitivities are regarded as more important than higher specificity values (Vatta *et al.,* 2001).

Positive predictive value, which is the proportion of samples with positive test results that are correctly diagnosed, was 91.7% in our report. This signifies that immunoperoxidase technique can correctly detect *T. gondii* in 91.7% of positive cases. This shows a high level of agreement between PCR and immunoperoxidase technique in the detection of *T. gondii.* This is similar to the 92% agreement reported by Sinn *et al.* (2017) between PCR and immunoperoxidase technique in the diagnosis of breast cancer.

We conclude that PCR is more sensitive than immunoperoxidase technique in the diagnosis of toxoplasmosis. However, with the high PPV and sensitivity of 84.6%, immunoperoxidase technique can be used as a screening tool in the detection of *T. gondii,* where PCR is not available, or in poor resource settings where the cost of analysis is a determining factor in the choice of diagnostic tool to use.

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Conflict of Interest
The authors declare that there is no conflict of interest.

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