

SEROLOGICAL SURVEY OF TOXOPLASMOSIS, NEOSPOROSIS AND BRUCELLOSIS AMONG CATTLE HERDS IN OYO STATE, SOUTH-WESTERN NIGERIA

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## Abstract

**Background:** Several zoonotic diseases are known to constitute great impediment to livestock management and production worldwide, especially in developing countries where control measures are largely non-existent. This study sets out to investigate the occurrence of toxoplasmosis, neosporosis and brucellosis among cattle herds in Oyo State, southwest Nigeria.

**Materials and Methods:** A cross-sectional survey to screen for antibodies to *Toxoplasma gondii*, *Neospora caninum* and *Brucella abortus* was conducted among 174 cattle in 17 herds. Sera obtained from the cattle were screened for antibodies to *Toxoplasma gondii* and *Neospora caninum* using enzyme-linked immunosorbent assay (ELISA) and for *Brucella abortus* antibodies using Rose Bengal test and Competitive Enzyme Linked Immunosorbent Assay (cELISA).

**Results:** Overall, herd level prevalence of 52.9%, 23.5% and 23.5% as well as individual prevalence of 7.5%, 3.4% and 3.4% was obtained for toxoplasmosis, neosporosis and brucellosis, respectively. Antibodies to *T. gondii*, *N. caninum* and *B. abortus* were detected in 2 of the 17 herds, *T. gondii* and *N. caninum* in 4 herds, and *T. gondii* and *B. abortus* in 4 herds. Statistically significant association was only found between seropositivity to *T. gondii* antibodies and sex ( $p < 0.05$ ).

**Conclusion:** Our results showed that toxoplasmosis, neosporosis and brucellosis are prevalent among cattle herds screened in the study area. Considering the potential impact of these diseases on livestock management and production, extensive surveillance is necessary for development and implementation of effective control and prevention strategies.

**Keywords:** *Toxoplasma gondii*, *Neospora caninum*, *Brucella abortus*, cattle, herd, Nigeria

## Introduction

*Toxoplasma gondii*, *Neospora caninum* and *Brucella abortus* are worldwide known abortifacients. These agents are not only detrimental to the reproductive capacity of livestock but also indirectly impact the livelihood of humans.

*Toxoplasma gondii*, an apicomplexan protozoa and the causative agent of bovine toxoplasmosis, is a zoonotic parasite with a worldwide distribution. The domestic cats and wild felids are the definitive hosts while various animal species (e.g. mammals, rodents, and birds) can serve as intermediate hosts (Dubey, 1986). *T. gondii* can be transmitted to animals and humans by the ingestion of food or water contaminated by oocysts shed from cat. The ingestion of tissue cysts in meat can also serve as infection source for humans (Tenter et al., 2000). There are several reports on the occurrence of *T. gondii* infection in cattle worldwide (Inpankaew et al., 2010; Dubey and Jones, 2008; Nematollahi and Moghddan, 2008; Klun et al., 2006; Ogawa et al., 2005; Esteban-Redondo et al., 1999), but there is still paucity of information on the role of *T. gondii* in reproductive failures in cattle. This is contrary to the situation in sheep where the parasite has been reported as a major cause of reproductive disorders such as embryonic death, abortion, stillbirth, and birth of weak animals (Masala et al., 2003; Dubey, 2009).

*Neospora caninum*, the etiologic agent of bovine neosporosis is also an apicomplexan protozoan parasite like *T. gondii*. Neosporosis is now known worldwide as one of the major causes of abortion, neonatal mortality and reduced milk production in both dairy and beef cattle (Hernandez et al., 2001; Dubey and Schares, 2011). The domestic dog (*Canis lupus familiaris*) along with gray wolves (*Canis lupus*) and coyotes (*Canis latrans*) serve as the definitive hosts for *N. caninum* and transplacental transmission is considered the most important route of infection in cattle, although the parasite can be acquired through the ingestion of oocysts shed from definitive hosts (Trees and Williams, 2005; Dubey and Schares, 2011). Neosporosis-associated bovine abortion and neonatal mortality has been reported from several parts of the world (Dubey, 2003). The reported serologic prevalence of bovine neosporosis has been

demonstrated to vary between countries and regions, type of test used, and cut-off level used to determine the exposure (Dubey, 2003). The abortion caused by *N. caninum* may be epidemic or endemic (Wouda et al., 1999) and seropositive cows are more likely to abort than seronegative cows (Dubey, 2003).

*Brucella abortus*, the etiology of bovine brucellosis, is a highly infectious pathogenic bacterial organism that causes infertility, abortion and reproductive failure in cattle (Carvalho-Neta et al., 2010). Brucellosis spreads through contact with aborted fetuses, vaginal fluids, placentae, placental fluids and milk, as well as through congenital and venereal means (Franco et al., 2007). It is one of the major zoonotic diseases of livestock with worldwide occurrence (Pappas et al., 2006) that usually results in huge economic losses due to reproductive problems that include abortion, early calf death, infertility, low milk yield, orchitis, epididymitis, seminal vesiculitis and other problems. (McDermott and Arimi, 2002; Carvalho Neta et al., 2010).

The diagnosis of *T. gondii* and *N. caninum* can be carried out using ELISA, the indirect fluorescent antibody test (IFAT), agglutination tests and immunoblotting techniques. Several assays such as the Rose Bengal test (RBT), card test (CT), serum agglutination test (SAT), rapid plate test (RPT), 2-mercaptoethanol test (2-ME), rivanol test (RIV), Coombs test, complement fixation test (CFT), milk ring test (MRT) and more recent diagnostic assays such as the competitive ELISA (C-ELISA), indirect ELISA (I-ELISA), and lateral flow assay (LFA) are available for detecting antibodies to *B. abortus* in blood or milk (Ducrottoy et al., 2014). Molecular techniques can be used for the screening of *T. gondii*, *N. caninum* and *B. abortus*.

In Nigeria, while there are reports on the occurrence of abortifacient pathogens in cattle herds, there is however paucity of studies directed at investigating more than one abortifacient infection in cattle herds at a time. This study was therefore aimed at investigating the occurrence of *Toxoplasma gondii*, *Neospora caninum* and *Brucella abortus* infections in cattle herds within a farm settlement in Oyo state, southwest Nigeria to generate baseline data that can be used in control and prevention of the disease in the study area.

## Materials and Methods

### Study area

The study was conducted in the rural farm settlements located in Itesiwaju Local Government Area (LGA) of Oyo State, southwestern Nigeria which lies between latitude 8°30' N and 7°45' N, and longitude 3°00' E and 4°00' E. The state is situated in the forest zone of Nigeria, which also consists of the derived savannah areas that encourage livestock farming. The rural farm settlements in Oyo State (like most northern parts of Nigeria) are home to numerous Fulani herdsmen. Historically, the Fulani herdsmen are believed to have migrated there at different periods from Northern Nigeria, in search of pasture and water.

### Sampling and Data Collection

Each herd was surveyed differently, and samples were randomly collected from about 10% of the total animals in a herd. Hence, varying number of animals were screened in all. The breed, sex and age of all animals were recorded. For each animal, approximately 10 mL of blood was collected in 15 ml sterile tubes. The blood samples were allowed to clot and centrifuged at 3000 g for 5 minutes. Serum samples were then decanted and stored at -20°C until they were assayed. In all, a total of 174 samples belonging to 17 different herds were collected.

### Detection of *Toxoplasma gondii* and *Neospora caninum* antibodies

In-house ELISAs was used to screen the samples. Briefly, purified 30 kDa *T. gondii* surface antigen (TgSAG1; Hosseini et al., 2009) was diluted in bicarbonate buffer (0.1 M, pH 7.2) and 120 µl/well of the diluted antigen (3 µg/well) used to sensitize ELISA plates for 1 h, 37°C and then washed three times with phosphate buffered saline (PBS), pH 7.2, supplemented with 0.05% (v/v) Tween® 20 (Serva, Heidelberg, Germany) (PBST) followed by a blocking step with PBST supplemented with 20% normal horse serum (Biochrom, Berlin, Germany) (SMB; 30 min, 37°C). The plate was emptied and 100 µl of controls and each serum sample, diluted in SMB, was added. The plates were washed three times with PBST. Species-specific conjugate (rabbit anti-cattle IgG (H + L)-POD; Dianova, Hamburg, Germany) was diluted 1:4000 in PBST supplemented with 1% (v/v) normal horse serum (Biochrom AG, Berlin, Germany). 100 µl of the diluted conjugate was added to each well and incubated (30 min, 37 °C). Subsequently, the plates were washed three times with PBST and twice with distilled water. 1% tetramethylbenzidine (TMB) with 0.012% (v/v) H<sub>2</sub>O<sub>2</sub> was added to each well. After 15 min at 37°C, the reaction was stopped by addition of 50 µl 4 N H<sub>2</sub>SO<sub>4</sub> and the optical density (OD) in each well read at 450 nm. Sample (S), Positive (PC) and negative control (NC) cattle sera were tested in duplicate in each plate. ELISA index values (I) were calculated for each sample (S) based on the means of two OD values obtained from an ELISA Reader using the formula  $I_S = (OD_S - OD_{NC}) / (OD_{PC} - OD_{NC})$ .

The procedure for *N. caninum* ELISA was carried out as previously done by Schares et al. (2009). The protocol is similar to the *T. gondii* ELISA except for the use of *N. caninum* surface antigen (NcSAG1) in place of *T. gondii* surface antigen (TgSAG1) and the use of a different conjugate.

### Detection of Brucella abortus antibodies

The Rose Bengal test (RBT): The RBT antigen consisting of standardized *B. abortus* antigen, sourced from the Veterinary Laboratories Agency, Surrey, United Kingdom was used to carry out the test (Alton et al., 1988). Briefly, equal volumes (30µl) of antigen and test sera including the positive control sera were mixed thoroughly on a plate using a stick applicator and the plate was rocked for 4 minutes. The appearance of agglutination within two minutes was scored (++) , while the agglutination after one minute was scored 1+ (+). The absence of agglutination after 4 minutes was scored negative (-).

Competitive Enzyme-linked Immunosorbent Assay (cELISA): The cELISA kit was sourced from the Veterinary Laboratories Agency, Surrey, United Kingdom. The reagents in the kit were reconstituted and the test was conducted according to the manufacturer’s instructions. The kit contained control sera (i.e. positive and negative controls), diluting buffer, conjugate, washing solution, chromogen and stop solution. The optical density (OD) was measured at 450nm using a microplate ELISA reader (Intertek Multiscan M11®). A positive/negative cut-off was calculated as 60% of the mean of the OD of the control wells. Samples in wells with OD values equal to or less than the cut-off point were scored positive while those above were scored negative.

### Statistics

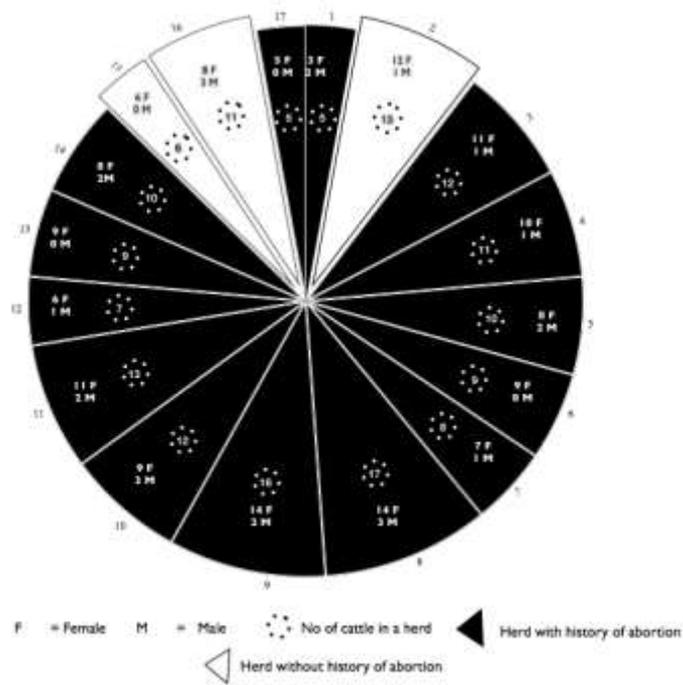
Data entry and analysis were performed using the statistical software package SPSS for Windows. Data were analysed to determine the association between seropositivity and breed, sex as well as age. Group differences were tested using Chi-square statistics for categorical variables. All tests were two-tailed and statistical significance was set at p<0.05.

### Results

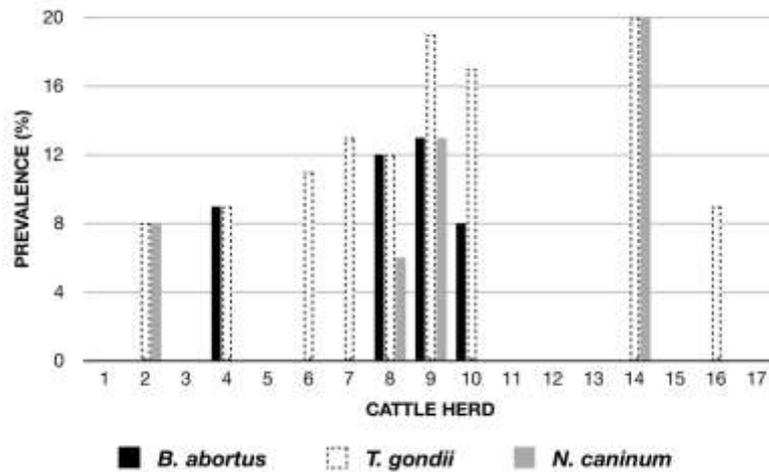
The distribution of cattle by herds, sex and history of abortion in the 17 herds is shown in Figure 1 while seroprevalence results for all diseases and distribution of seropositive cattle by age and sex is summarized in Table 1. Toxoplasmosis had the highest seroprevalence (7.5%) while neosporosis and brucellosis had seroprevalence of 3.4% each. Serum positive for *T. gondii* with *N. caninum* and *T. gondii* with *B. abortus* were found in one (0.58%) and two (1.2%) cattle, respectively.

**Table 1:** Prevalence of antibodies to *T. gondii*, *N. caninum* and *B. abortus* in studied cattle herds in Oyo State, Nigeria

Parasite	No positive/tested	Prevalence (%)	No positive (%)			
			Female (N =150)	Male (N = 24)	Adult (> 1yr) (N = 172)	Young (< 1yr) (N = 2)
<i>Neospora caninum</i>	6/174	3.4	4 (2.7)	2 (8.3)	6 (3.5)	0 (0)
<i>Toxoplasma gondii</i>	13/174	7.5	8 (5.3)	5 (20.8)	12 (7.0)	1 (33.3)
<i>Brucella Abortus</i>	6/174	3.4	6 (4)	0 (0)	6 (3.5)	0 (0)
<i>T. gondii</i> & <i>N. caninum</i>	1/174	0.58	1 (0.7)	0 (0)	1 (0.58)	0 (0)
<i>T. gondii</i> & <i>B. abortus</i>	2/174	1.2	2 (1.3)	0 (0)	2 (1.2)	0 (0)



**Figure 1:** Pie chart showing the distribution of cattle, sex and history of abortion in the 17 herds of cattle.



**Figure 2:** Seroprevalence of *T. gondii*, *N. caninum* and *B. abortus* in the 17 herds of cattle.

At the herd level (Figure 2), only nine (52.9%) of the 17 herds tested positive for at least one disease (*T. gondii*) while cattle in the remaining 8 herds tested negative to all the diseases. Antibodies to the 3 diseases were detected in only two (11.8%) of the herds. *T. gondii* antibodies only were found in three (17.6%) herds while. *N. caninum* and *B. abortus* were detected in four (23.5%) of the herds. The seroprevalence of *T. gondii* and *N. caninum* was highest in herd 14 and *B. abortus* was at the highest level in herd 9.

Seropositivity to toxoplasmosis, neosporosis and brucellosis was higher in female and adult cattle than in male and younger ones. Statistically significant association was only found between seropositivity to *T. gondii* antibodies and sex ( $p = 0.0197$ ; OR= 0.214; CI=0.064 – 0.722).

## Discussion

The potential impact of abortifacient pathogens on livestock management and production cannot be overemphasized. Hence, there is the need to constantly screen for abortifacient infections to ensure the production of cattle with good reproductive performance. The present study was carried out to investigate the occurrence of antibodies to *B. abortus*, *N. caninum* and *T. gondii* in some cattle herds in Oyo State, southwestern Nigeria.

The seroprevalence of *T. gondii* infection obtained in this study (7.5%) was higher than those reported in Tanzania (3.6%, Luuk et al., 2009) and Iran (0%, Hashemi-Fesharki, 1996). However, the finding of the present study is similar to those obtained in France (7.8%, Gilot-Fromont et al., 2009) and Malaysia (7.9%, Chandrawathani et al. 2008) but lower than previous reports from Brazil (71.0%, Santos et al., 2009) and Serbia (76.3%, Klun et al., 2006). The low seroprevalence of *T. gondii* antibodies in the present study compared with other places with high prevalence may be due to low population of cats in the studied area. Other factors such as method of detection, breed susceptibility, management practices and environmental contamination may also be responsible for the variable seroprevalence. According to our result, exposure of cattle was more to *T. gondii* than *B. abortus* and *N. caninum*. This finding, from the epidemiological perspective, may suggest a wider spread of *T. gondii* infection in cattle population in this region. *Toxoplasma gondii* has been reported as one of the most polyxenous parasites known to date and causes congenital disease in the intermediate host, although the infection in cattle has been suggested to be of less importance than in pigs, sheep and goats (Tenter et al., 2000). More studies are needed to study the importance of bovine toxoplasmosis in reproductive failures of cattle in Nigeria.

For *N. caninum*, a seroprevalence of 3.4% in the present herd-based study is similar to 2.8% obtained in our previous study on 500 local cattle from the abattoir (Ayinmode and Akanbi, 2012). In another study, we detected antibodies to *N. caninum* in 4% of imported cattle raised under intensive management in Nigeria (Ayinmode, 2013). Although all the values obtained so far in Nigeria are lower than those obtained in Iran (32%, Youssefi et al., 2009), Western Romania (27.7%, Imre et al., 2012) and Senegal (17.9%, Kamga-Waladjo et al., 2010), it is, however, worth noting that the detection of *N. caninum* in 23.5% of the herds in the present study along with previous findings may suggest that neosporosis is endemic in Nigeria and there is need to educate farmers on how to prevent and control the disease. Prevention of dogs (the definitive host) from contaminating pastures and feedstuff with faeces has been recommended as a major way of preventing neosporosis (Hernandez et al., 2001; Dubey and Schares, 2011).

The overall 3.4% individual animal and 23.5% herd seroprevalence obtained for brucellosis in this study indicate that the disease is prevalent among the cattle herd screened. This may be attributed to the fact that a brucellosis control programme is non-existent in Nigeria and vaccination of cattle against brucellosis is not done (Rikin, 1988; Ducrotoy et al., 2014). Also, since animal movements are not regulated, there is a high possibility of inter-cattle herd transmission between infected and un-infected animals grazing on common pasture and/or watering points. Furthermore, the nomadic system of animal husbandry practiced by Fulani pastoralists in Nigeria has been implicated as a major risk factor for brucellosis (Mai et al., 2012; Ducrotoy et al., 2014).

All the seropositive animals were females and adults, a finding that is consistent with those of other authors (Kubuafor et al., 2000; Mai et al., 2012; Junaidu et al., 2008; Junaidu et al., 2011) who reported higher seroprevalence of brucellosis in females and adult animals. This could be attributed to the fact that brucellosis is a reproductive disease that is also directly linked to maturity. Sexually mature and pregnant cattle are more susceptible to *Brucella* infection than sexually immature ones (Chimana et al., 2010). For reproductive reasons, female animals are left in the herd for longer periods of time thereby increasing their chances of contracting the infection due to prolonged exposure to the organism. Such animal could also serve as a source of infection for other susceptible animals. Again, vulnerability of animals to *Brucella* infection has been reported to increase with increase in breeding age (Chimana et al., 2010; Radostits et al., 1994).

This study observed that *T. gondii* and *N. caninum* infection was highest in herd 14 and *B. abortus* in herd 9. The reason for this discrepancy is not known, although it may be associated with differences in management practices and other intrinsic factors of the herds. Further studies are needed to elucidate the risk factors for these diseases in herds in studied area.

In this study, we observed that despite the fact that all the herds have history of abortion, there were herds without a single animal positive for toxoplasmosis, neosporosis and brucellosis. This suggests that there could be other causes of reproductive problems in these herds. Further studies are needed to investigate the presence of other abortifacient pathogens like *Campylobacter* sp., *Leptospira* sp., Tritrichomonosis and viruses in all the herds. These infections might be responsible for the low number of calves (only 3 out of 174 cattle) in the herds.

In conclusion, this study provides evidence of exposure of cattle to co-infection of abortifacient agents in the study area and suggests that seropositivity to *T. gondii*, *N. caninum* and *B. abortus* does not necessarily correlate with reproductive problems in the herds. Screening for all known abortifacient organisms is therefore recommended in herds for effective implementation of preventive and control measures against reproductive losses in cattle.

## Acknowledgement

We thank Andrea Bärwald for her excellent technical assistance. We express our thanks to the DAAD fellowship for sponsoring Dr. Ayinmode to conduct part of this study at the Friedrich Loeffler Institute, Insel Riems, Germany.

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