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RESEARCH ARTICLE

TOXOPLASMOSIS IN BROILERS FROM DAKAR - SENEGAL: PREVALENCE AND ASSOCIATED FACTORS

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Abstract

Toxoplasmosis is one of the most common zoonosis worldwide. Caused by the protozoan parasite *Toxoplasma gondii*, it has estimated that up to a third of the human population worldwide is infected with it. Humans are infected by ingesting oocysts shed by cats or by eating undercooked meat containing cysts. *T. gondii* can cause serious illness to the fetus during a congenital infection and fatal in immunocompromised patients too. In addition, it infects a wide range of animals, including mammals and birds. In birds, all species can be infected with various signs. Little is known about the prevalence in short-cycle species (broilers). However, due to their feeding habits (birds are pecking and their food is usually soil), the prevalence in birds could be a good indicator of the prevalence of parasite oocysts in soil. Thus, to verify environmental contamination, the prevalence of *T. gondii* in 459 short-cycle birds (broilers) was determined in Dakar, Senegal. The diagnostic test used was the High Sensitivity Direct Agglutination test (ADHS). The results showed the presence of antibodies against *T. gondii* in 29 (6.36%) of the broilers *Gallus domesticus*. This study is the first of its kind to investigate *T. gondii* in short-cycle domestic poultry in Senegal. Other studies should be carried out with additional and more reliable techniques on this environmental aspect in order to measure the real parasite load and the risks that this could generate.

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Introduction:-

Toxoplasmosis is a protozoonosis caused by *T. gondii* protozoan, which can infest all homeotherms. The felidae are the only known definitive hosts. They disseminate a large number of oocysts in their faeces which, after sporulation maintain their infectivity for several months in the environment. These oocysts are the source of contamination of slaughtered animals. In the intermediate host, the parasite encysts in tissues such as the brain, heart, or skeletal muscles. People usually become infected by ingesting the oocysts or by eating undercooked meat or raw vegetables

containing the cysts. Toxoplasmosis is a public health problem. It is generally asymptomatic, however some clinical forms can be observed mainly in immunocompromised individuals or during congenital transmission. Numerous epidemiological investigations have incriminated meat as an important vector of contamination and poultry as a sentinel and indicator of environmental contamination. Indeed, if it is complicated to search for the toxoplasm directly in the soil, it is much easier through a concentration effect to search for them in pecking animals therefore feeding on the ground such as poultry.

Avian toxoplasmosis has been known for decades and all birds, even wild ones should be considered as potential sources of toxoplasms [1, 2]. Even if little is known on the prevalence of the parasite in migratory species, birds are able to transmit the toxoplasm over large geographic distances by the phenomenon of migration [2].

Sedentary species that have been domesticated are used as bio-indicators of soil contamination by oocysts, especially in countries where climate conditions are very favorable for the survival of oocysts [3].

Most of the studies carried out only focus on traditional herds and show medium to high seroprevalences of up to 65% in herds in South America and 40-50% in Africa [4, 5, 6]. On the contrary, 6.2% and 16.9% of seropositive chickens were found respectively in Mexico [7] and in the United States [8].

The epidemiological role of the poultry seems to be that of a sentinel. Indeed, those that peck outside are an indicator of environmental contamination in toxoplasms and therefore a good source to search for environmental strains of *T. gondii*. Finally, according to some authors, the high frequency and the prevalence observed in the poultry could lead to the conclusion of a risk to human contamination [9, 10, 11, 12]. Due to the relatively short production cycle of broilers, their seroprevalence is generally not studied.

In Senegal, the high frequency of cats in homes, streets and even henhouses (personal survey) suggests a possibility of chickens' contamination regardless of the production cycle (short or long). However, there is a lack of data to capture these details. In order to overcome this problem of lack of data and to verify the environmental contamination, which remains a potential, risk for human population, this study has set its general objective to determine the seroprevalence of toxoplasmosis in Dakar short-cycle birds.

Material And Methods:-

Areas and study period:

The main market of Guédiawaye was the preferred site for this study, which took place over three months from December 2015 to February 2016. Indeed, the choice of the site is justified by the fact that it is the central market where the majority of women buy chickens and other cooking ingredients.

Calculation of the required sample size:

The Win Episcope version 2.0 software was used to calculate the sample sizes. A sample of 50 poultry per seller was determined this, for a slaughter rate varying between 50 and 100 per day per seller, with an expected prevalence (Patt) of 5% and a risk of error (d) of 5%.

Survey:

Farmers who are at the same time chickens sellers at the Guédiawaye market were contacted. A survey sheet was completed by each of them mentioning the origin of the animals and the method of breeding, the type of building, the presence or not of cats and other animals on the site.

Samples collection:

At the Guédiawaye market, the blood of the chicken was collected from farmers-sellers who signed up for the study. The animals' blood was collected randomly as per the time of bleeding and as their entry into the bleeding room. The blood samples were then sent immediately to the laboratory where the sera collected after clot shrinkage were stored in a freezer at -20°C for later serological testing.

Serological techniques:

All collected sera were analyzed by the High Sensitivity Direct Agglutination (ADHS) test with the Reims antigen. In total, four dilutions (1/20, 1/40, 1/100, 1/800) were made.

Principle of the A.D.H.S.

The principle of the test is based on the ADHS of formalin toxoplasms (antigen) by specific antibodies (IgG) present in the serum of the infected animals. The antigen is prepared according to Desmonts and Remington (1980) protocol.

The test is carried out on series of dilutions of serum treated with 2-mercapto-ethanol (2ME) which destroys IgM and therefore only detects IgG. Very sensitive (positivity threshold = 4 IU), it can be used in screening and titration. In general, bird sera are tested at a dilution of 1/10 to 1/80 or 1/160 and the cut-off point is 1/10.

The reading is taken after 5 to 24 hours of incubation at room temperature.

Reading:

For a positive reaction, a haze of agglutinated toxoplasma forms and covers more than half of the bottom of the well. For the negative reaction, the formulated toxoplasms sediment at the bottom of the well in the form of a whitish (or reddish depending on the diluent used) button or the veil covers less than 50% of the well (Fig1).

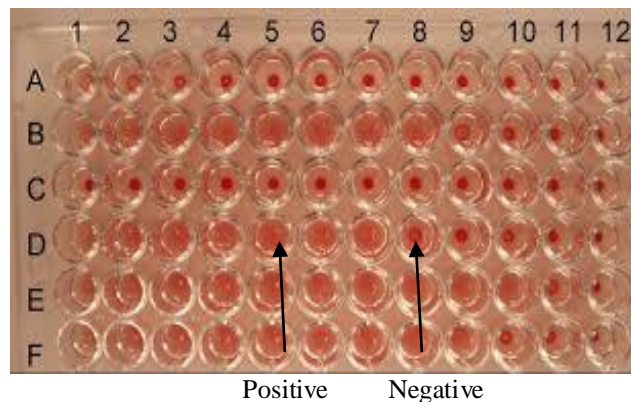


Figure I:- ADHS reading plate.

Results:-

A total of 459 birds were collected. The overall prevalence was 6.32% all vendor combined.

In total, out of the five (5) main broilers breeder-sellers at the Guédiawaye Market, three (3) agreed to participate in the study. Four hundred and fifty-nine (459) broilers broilers were collected from these vendors, of which 156 were from vendor 1, 146 from vendor 2 and 156 from vendor 3.

• Overall prevalence and by vendor:

Of the 459 broiler samples collected, 29 were in contact with the parasite resulting in a prevalence of 6.32% with 95% CI = (5.41-7.21). The prevalence was higher (7.53%) in vendor 2 (V2) and vendor 3 (V3: 7.01%) compared to vendor 1 (V1: 4.49%) (Fig 2).

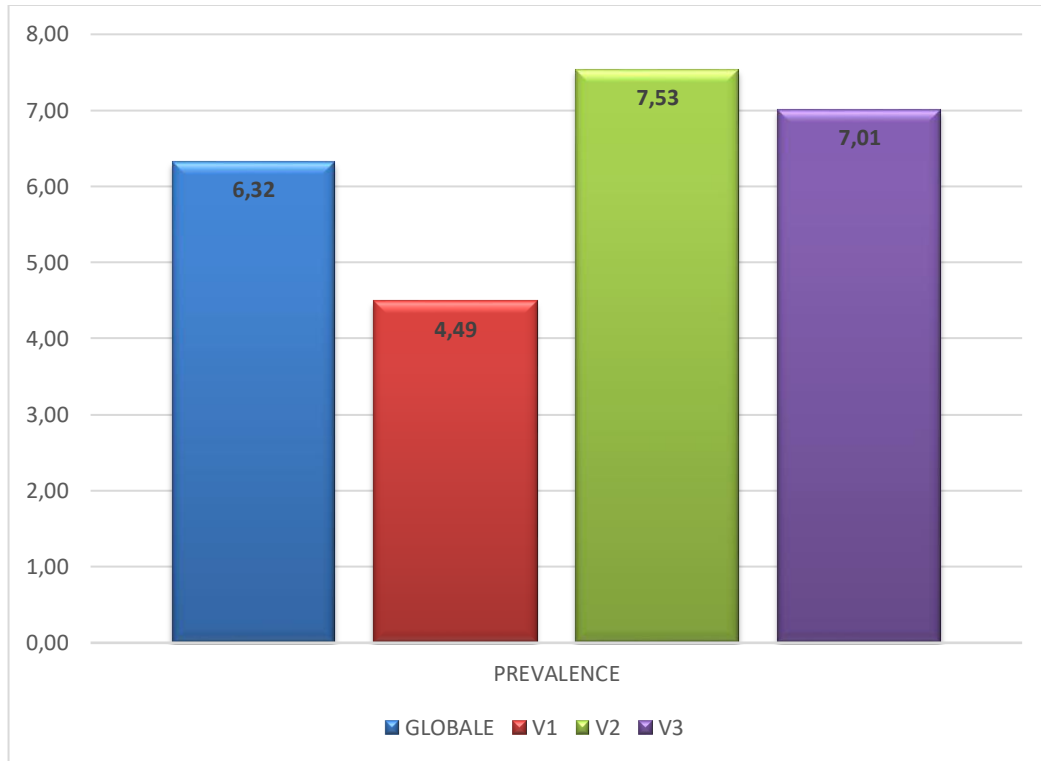


Figure II:- Overall prevalence and by vendor of broilers.

• Prevalence by dilution:

Four (4) dilutions has carried out, namely 1/20, 1/40, 1/100 and 1/800 with a positivity threshold set at the 1/10 dilution. The positivity was more evident in the first two dilutions (1/20 and 1/40) with a prevalence of 7.63% and 3.70% respectively compared to the last two (1/100 = 1.53% and 1/800 = 0.65%) (Fig3).

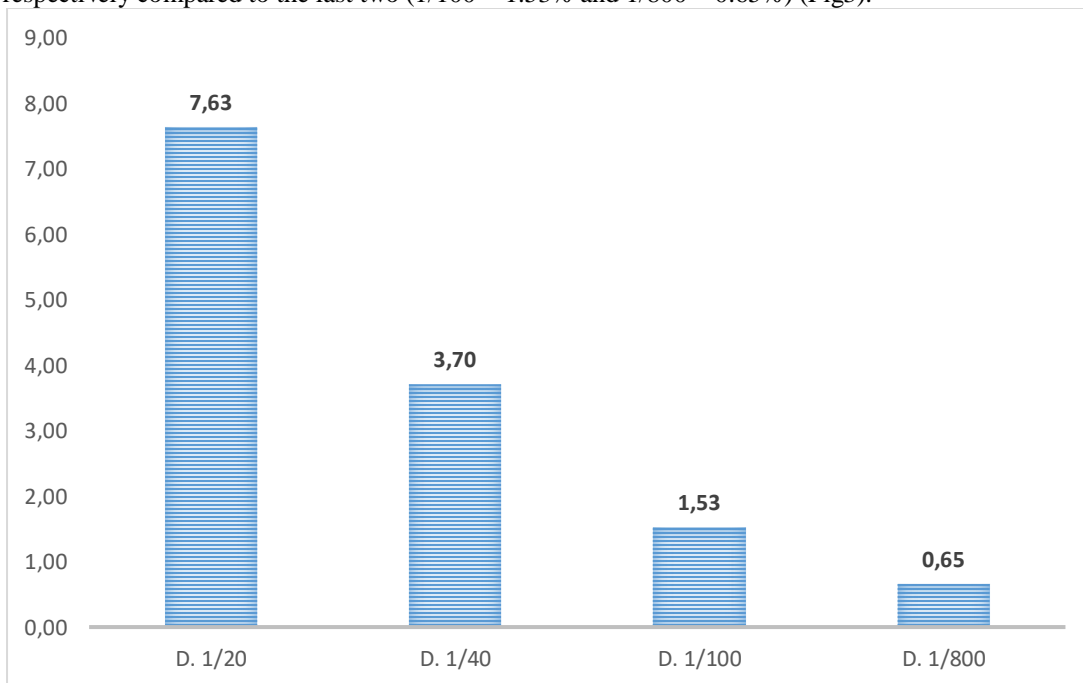


Figure III:- Prevalence as per the dilutions in the broilers.

D. = dilution

• **Factors associated with the seroprevalence:**

All the animals came from various origins. However, the vendors 2 and 3 had wires meshes buildings with very easy access to the kittens unlike the vendor 1. They also had beside the broilers other types of animals on the farm. Hence, they had higher prevalences compared to the seller 1.

Table I:- Environmental factors (EF) and bi-variate analysis.

FE	Presence of cat	Type building	Type of soil	Origine of animals	Mode of breeding	Presence Of other animals	n	Pr	p-value	OR (IC _{95%})
V1	No	Closed	Sandy	Diverse	Intensive	No	156	4,49	0,33	0,58 (0,18-1,68)
V2	Yes	Wire mesh	Sandy	Diverse	Intensive	Yes*	146	7,53	Réf.	Réf.
V3	Yes	Wire mesh	Sandy	Diverse	Intensive	Yes**	157	7,05	0,8	0,90 (0,34-2,37)

*Sheeps, guinea fowls, traditional poultry

**Pigeons, guinea fowls, traditional poultry

Discussion:-

During this study, various problems were encountered notably the refusal of sellers to collaborate and the difficulty of collecting blood due to the conditions under which the animals were slaughtered. However, these difficulties did not prevent us from achieving our goals.

Poultry are used as bio-indicators of soil contamination by the oocysts, especially in countries where the climatic conditions are very favorable for the oocyst survival [3]. According to Dubey [13], depending on the source of the chicken, the prevalence ranged from 2-100%.

The prevalence obtained in caged chickens (intensive breeding) in this study was 6.32% (29/449). The majority of studies carried out relate only to traditional breeding.

However, our results are in agreement with those of a study carried out in the Northeast of China in which the author using the agglutination technique (MAT) found a prevalence of 6.23% (19/305) in caged chickens [14].

However, lower prevalence is than ours have been found in caged chickens elsewhere: in Japan in the prefecture Gifu 0% (0/72) [15]; in southern China: 4.1% (10/244) [16] and in the North-Eastern China 5.6% (25/450) [17].

In over side, higher values than ours were also recorded as it was the case in Egypt where the author found 18.7% (28/150) at the slaughterhouse using the MAT technique [18], in Thailand [13], 64 % with the use of IFA as a diagnostic test. This variability in the prevalence between studies could be attributed to various factors such as the diagnostic test used, the environment, the hygiene and the access of cats to the breeding premises.

However, compared to work done in traditional chickens, our results obtained in poultry flesh (6.36%) substantiate with those obtained from traditional poultry in Mexico [7]. This could be explained by the fact that these animals have the same feeding pattern.

Elsewhere, higher values were observed for traditional poultry: (12.5%: 10/80) (24.2% (81/330), 24.4% (24/98), 30% (6/20) respectively in Italy, Vietnam, Indonesia, and Poland [19], 38.4% and 40.4% in Egypt [4, 20], 64% (41/64) in Ghana [19].

The breeding model and the life cycle of the two groups of birds could explain this variation. Indeed, the broilers poultry are raised in an intensive mode (in cages and fed with industrial feed) with a very short life cycle ranging from 30 to 45 days depending on the farms while traditional poultry are raised extensively with a much longer life

cycle. The latter, therefore have time to meet the parasite, especially as they are usually wandering around looking for food.

This difference could also be due to the breeding and the geo-climatic conditions affecting sporulation and survival of oocysts in the environment.

Furthermore, all these prevalences demonstrate the ubiquity of avian toxoplasmosis.

Conclusion:-

The seroprevalence of 6.23% has highlighted the existing contact between the birds and the toxoplasma in Senegal.

Considering the feeding manners of these birds, these results demonstrate the presence of *T. gondii* in the environment. The ministries in charge of animal and human health should accept the promotion of this type of health surveillance.

Control measures should be taken in African countries, which share the same way of living, and environmental studies should be carried out to assess the parasitic load in the environment.

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