

TOXOPLASMOSIS-A DISEASE WITH HIGH EPIDEMIOLOGICAL RISK IN HUMANS AND ANIMALS

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Abstract

Infections produced by the protozoan *Toxoplasma gondii* are widespread in humans and animals. Due to its lack of host specificity, this parasite is able to infect a large number of hosts as well as different cell types. Although toxoplasmosis is the most reported parasitic zoonosis in Europe, the incidence of the disease in humans and the presence of the parasite in animals, food and water is underestimated. If acquired as an acute infection during pregnancy, *Toxoplasma gondii* infection can have serious adverse effects on mothers, foetuses and newborns. Latent toxoplasmosis also causes a variety of pathologies and has been linked to serious adverse effects on pregnancy. The study was conducted over a 2-year period, 2019-2020, in the Parasitology Clinic of the Faculty of Veterinary Medicine, Iasi, following the prevalence of reported cases of toxoplasmosis in cats. Thus, out of 33 tests worked, no case of toxoplasmosis was recorded in cats, all serological tests being done upon request. During 2020, 226 AB. ANTI TOXOPLASMA GONDII- IgM (ELISA) tests were performed in the Praxis laboratory, of which only 15 were positive. All positive tests were identified only in women, of which 10 in the age category 25-34 years, 4 in the age category 35-44 years and 1 case in the age category 15-19 years. In the Praxis laboratory during 2020, 220 more AB. ANTI TOXOPLASMA GONDII- IgG (ELISA) tests were performed, out of which 72 positive cases were identified, 5 being positive in males in the age categories 0-12 months, 1 year and 15-19 years, and the remaining 67 were identified in women in the following age categories: 0-12 months, 15-19 years, 20-24 years, 25-34 years, 35-44 years, 45-54 years and 55-64 years. The lack of positive cases in animals during the 2-year study, but the high number of positive cases in humans during a single year, shows the major public health importance of the study, as this very serious disease in pregnant women and immunosuppressed people is under-diagnosed in veterinary medicine.

Keys words: *Toxoplasma gondii*, parasitic zoonosis.

Despite the fact that toxoplasmosis is the most reported parasitic zoonosis in Europe, the incidence of the disease in humans and the presence of the parasite in animals, food and water is underestimated (EFSA, 2007). In order to understand the molecular epidemiology of toxoplasmosis, it is necessary to study strains from different geographical areas. The first studies on *Toxoplasma gondii* strains were obtained in humans and domestic animals, mostly from the USA and Europe, based on single-cell analysis. No atypical or recombinant strains could be identified in the studies. The study of multiple markers can accurately determine circulating genotypes, because strains belonging to specific genotypes, but also non-specific strains showing atypical alleles can be identified by means of multilocus analysis. In a study of a large number of strains isolated from different geographical areas, four distinct populations were identified (LEHMANN et al., 2006). One population was identified on all continents except South America, two populations

were restricted to South America, the fourth population had a ubiquitous distribution. The current distribution of *T. gondii* can be explained by migration from South America to Eurasia and from Europe to North America and Southeast Asia (LEHMANN et al., 2006). KHAN et al. (2007) determined 11 haplogroups in 46 strains of *T. gondii*. These haplogroups had a different geographical distribution. This high diversity of *T. gondii* is probably due to host diversity which is much higher in wild host populations (AJZENBERG et al., 2004). Five countries in Europe (Austria, Belgium, France, Slovakia and Slovenia) have recorded active surveillance of congenital cases with mandatory screening of pregnant women. Twenty-two EU/EEA Member States reported toxoplasmosis data to TESSy (21 EU Member States plus Iceland). Denmark, Italy, the Netherlands, Norway, Portugal and Sweden did not have a toxoplasmosis surveillance system. Spain did not have national surveillance and could not provide any estimate for population coverage, so no

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notification rate was calculated. One Member State used the most recent case definition from 2018, eight Member States used the 2012 case definition, nine countries used the 2008 case definition, one Member State reported using the 2002 case definition, two used a different case definition, and did not specify. France regularly reports the highest number of congenital toxoplasmosis cases, most likely due to the sensitivity of its surveillance system that includes screening pregnant women, follow-up of those detected with infestation during pregnancy, and laboratory confirmation of any congenital toxoplasmosis cases detected during the process, including asymptomatic cases (<https://www.ecdc.europa.eu/en/toxoplasmosis>).

Congenital toxoplasmosis in the EU/EEA increased between 2012-2015, mainly due to reporting by France, which accounted for up to 90% of all cases reported during that period. Even without symptoms, pregnant women can transmit toxoplasma infection to the fetus, which can lead to miscarriage, perinatal death or congenital infection with severe malformation affecting the eyes and brain. Infection in people with low immunity tends to severely affect the central nervous system, but other organs may also be affected. Such patients may require prolonged (sometimes lifelong) therapy. The cost of benefits of prenatal screening programmes have been debated due to the low prevalence of congenital toxoplasmosis in the EU/EEA and uncertainty about the effectiveness of prenatal treatment. A retrospective of the Austrian national prenatal screening programme study concluded that between 1992-2008, it saved social costs of more than €15 million per year and €258 million over 17 years. In France, 79% of maternal infections did not result in clinical symptoms in newborns and birth defects occurred in less than 1%. The authors attributed low morbidity and mortality to early diagnosis and treatment of maternal infections. Nanotechnology is currently being investigated as a tool to manage *T. gondii* infections, as well as the development of vaccines using the mRNA sequence encoding disease-specific antigens. These developments could prove useful in the diagnosis, treatment and possible prevention of congenital toxoplasmosis.

Congenital toxoplasmosis can result in severe disease in infected fetuses. The problem with congenital toxoplasmosis in the EU/EEA is that it cannot be evaluated because of the large differences between national surveillance systems, screening programmes and follow-up of pregnant women. However, regardless of national strategies for surveillance, it is important to strengthen prevention options for congenital toxoplasmosis. Pregnant women at risk of *T. gondii* infection should receive

information about exposure and prevention. Based on official reports, more than one billion people are estimated to be infected with *Toxoplasma gondii*, which is transmitted mainly through ingestion of food, water, vegetables and fruit contaminated with sporulated oocysts eliminated by cats or ingestion of vegetative cysts from raw or undercooked meat. The overall cumulative *T. gondii* seroprevalence was estimated at 35% and 59% in domestic and feral cats, respectively, using the random effects model. Seroprevalence was higher in Australia and Africa, where *T. gondii* seropositivity in domestic cats was of 52% and 51% respectively. The lowest seroprevalence was estimated in Asia 27%. The seroprevalence of *T. gondii* in feral cats was of 74% in Africa, 67% in Asia, 67% in Europe and 66% in South America. Infection acquired in the first trimester of pregnancy by untreated women causes congenital infection in 15-25% of newborns. If infection occurs in the second or third trimester of pregnancy, fetal infection occurs in 25-65% of cases. In 70-90% of infected babies, no clinical signs of disease appear, but in a proportion of them clinical lesions manifest as they grow.

The clinical manifestations of the disease may be ocular (chorioretinitis, strabismus, blindness), neuro-psychological (psychomotor or mental retardation, epilepsy, microcephaly, hydrocephalus), haematological (thrombocytopenia, anaemia), other (hypothermia, pneumonia, diarrhoea).

Chronically, in animals the clinical picture is uncertain. In cats, in the case of a low degree of infestation, no clinical symptoms appear. In most cases, in all types of intermediate host infection toxoplasmosis does not manifest by means of clinical signs or is expressed by a mild form of adenomegaly. If infection occurs in pregnant females in whom natural immunosuppression is present, tachyzoites may cross the placenta and contaminate the fetus, leading to fetal death in early pregnancy, miscarriage, birth of a deformed fetus or death by sepsis before 2 months of age.

MATERIAL AND METHOD

The study aimed to determine the prevalence of toxoplasmosis in humans and animals in Iasi County, in order to demonstrate the need for the introduction of monitoring programmes for this protozoa in animals, being a disease underdiagnosed in veterinary medicine in Romania. The study was carried out at the Faculty of Veterinary Medicine in Iasi and at the Praxis medical tests laboratory.

The aim was to determine the prevalence of toxoplasmosis cases registered at the Faculty of

Veterinary Medicine during one year. Since the intestinal form of toxoplasmosis in cats (where oocyst formation occurs - the form of resistance in the environment, and which is the route of contamination of intermediate hosts) is most often asymptomatic or presents mild symptoms, serological diagnosis by detection of antigen and antibodies is recommended.

In the parasitology laboratory the diagnosis of toxoplasmosis is made by detection of antigen and antibodies using rapid veterinary tests - *Toxoplasma gondii* Ag, *Toxoplasma gondii* IgG and IgM respectively. WELLTEST *Toxoplasma gondii* Anti is a rapid immunochromatographic test for the qualitative detection of *Toxoplasma gondii* antigens in faecal samples, serum or plasma from animals. It is a rapid assay using the double layer lateral flow immunochromatographic method, sandwich format. Also, to aid in the diagnosis of acute infection it is recommended to follow the antibody titre in the dynamic by assaying for *T. gondii* specific IgG and IgM titres followed by a second assay 2-4 weeks later.

Maravet Toxoplasma Ab Rapid Test

The Maravet Toxoplasma Ab Rapid Test, which is a qualitative lateral flow immunochromatographic sandwich assay for the determination of *Toxoplasma* antibodies (CHW Ag)

Results of the epidemiological investigations performed at the Praxis laboratory using (table 1)

in animal blood, was also used. The principle of the Maravet Toxoplasma Ab Rapid Test is based on the lateral flow sandwich immunochromatographic reaction.

Cats presented to the Parasitology Clinic during 2020 with suspected toxoplasmosis or at a control examination were taken in the study to rule out the presence of the protozoan, and therefore, the risk of contamination of the human host, the owner being pregnant.

RESULTS AND DISCUSSION

Out of a total of 33 cats tested, for the removal of suspected toxoplasmosis, no case was confirmed positive. The majority of the samples evaluated, 26 in number, were from owners who wanted to exclude the presence of *Toxoplasma gondii* in the cat as the owner was pregnant. Suspected cases of toxoplasmosis (4 cases) were those with a digestive clinical picture, mainly diarrhoea with mucus, which did not respond to a usual antiparasitic treatment.

Of the 33 samples evaluated, there were no positive cases. The results of the epidemiological investigations carried out at the Praxis laboratory, Iasi county over a period of 1 year are presented as follows:

Table 1

AB. ANTI TOXOPLASMA GONDII- IgM (ELISA) assay/test

Name Age group	Total	Total negative	Total positive	Positive Sex M	Positive Sex F	Total Sex M	Total Sex F
0-12 months	7	7	0	0	0	6	1
1 year	3	3	0	0	0	3	0
2 years	0	0	0	0	0	0	0
3 years	0	0	0	0	0	0	0
4 years	1	1	0	0	0	1	0
5-9 years	1	1	0	0	0	1	0
10-14 years	3	3	0	0	0	1	2
15-19 years	4	3	1	0	1	1	3
20-24 years	21	21	0	0	0	0	21
25-34 years	136	126	10	0	10	1	135
35-44 years	44	40	4	0	4	1	43
45-54 years	3	3	0	0	0	2	1
55-64 years	2	2	0	0	0	1	1
65-74 years	1	1	0	0	0	0	1
75-84 years	0	0	0	0	0	0	0
85+ years	0	0	0	0	0	0	0
TOTAL	226	211	15	0	15	18	208

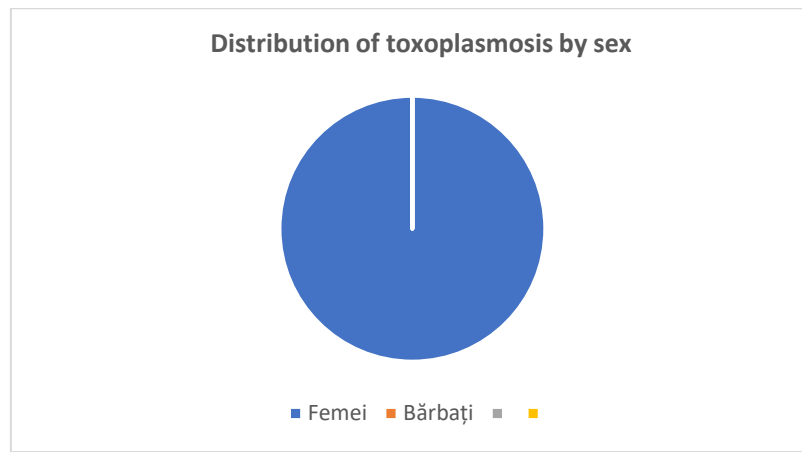


Figure 1 Distribution of toxoplasmosis by sex in human, women-men

As for the distribution of toxoplasmosis by sex, it is very unbalanced, with 100% of cases diagnosed in women. The result is explained by the fact that clinical toxoplasmosis in humans is influenced by the low immune status (*figure 1*). During pregnancy, testing for acute-phase IgM and IgG memory antibodies, respectively, is mandatory, as the disease can cause miscarriage and/or severe congenital malformations in the fetus. The absence

of positive cases of toxoplasmosis in the animals studied, but the presence of a considerable number of positive cases in humans, demonstrates that toxoplasmosis is a neglected disease of major importance to human health and is widespread worldwide.

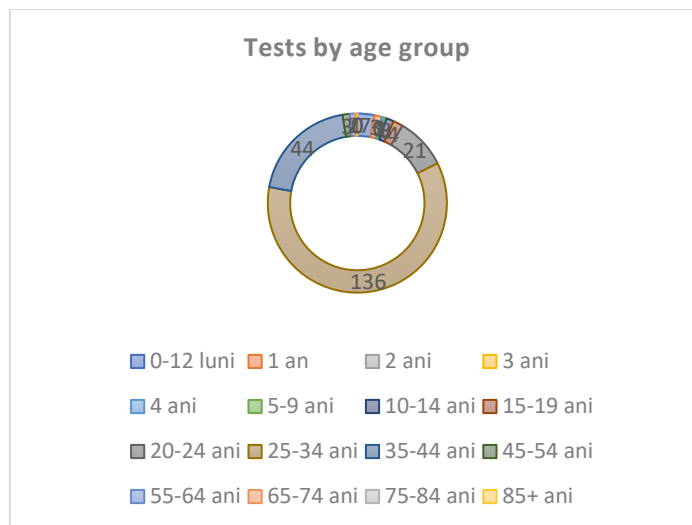


Figure 2 Tests by age group (months/year/years)

Out of a total of 226 tests performed for females and males, 60.17% (136) were performed in the age group 25-34 years, 19.46% (44) were performed in the age group 35-44 years, 9.29% (21) of the tests were performed in the age group 20-24 years, 3.09% (7) of the tests were performed in the

age group 0-12 months, and the rest of the tests were performed in the other age groups. The high proportion of testing in the 25-34 age group is strictly related to the number of pregnant women in this age group (*figure 2*).

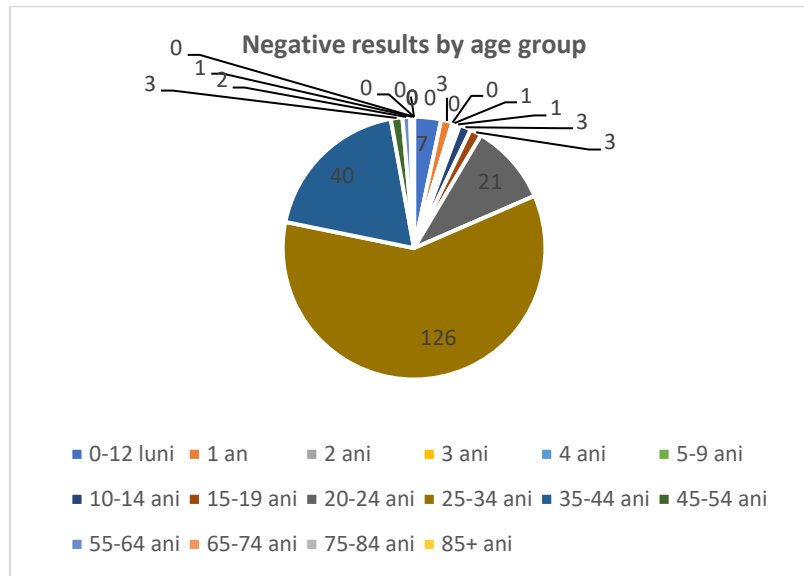


Figure 3 Negative results by age group

Out of a total of 211 negative tests performed for females and males, 59.71% (126) were performed in the age group 25-34 years, 19.95% (40) of the negative tests were performed in the age group 35-44 years, 9.95% of the negative

tests were recorded in the age group 20-24 years, 3.31% (7) were performed in the age group 0-12 months, and the rest were performed in the other age groups (*figure 3*).

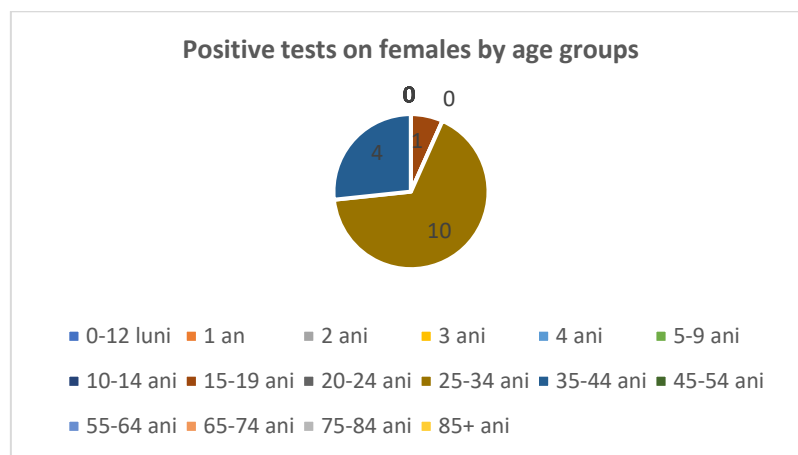


Figure 4 Positive results in females by age group

Of the 15 confirmed cases, 66.66% (10) were in the age group 25-34 years, 26.66% (4) were found in the age group 35-44 years, and 6.66% (1) were found in the age group 15-19 years. The high

proportion of positive cases in the age group 25-34 years is directly proportional to the high number of cases tested at this age, being a mandatory test during pregnancy (*figure 4*).

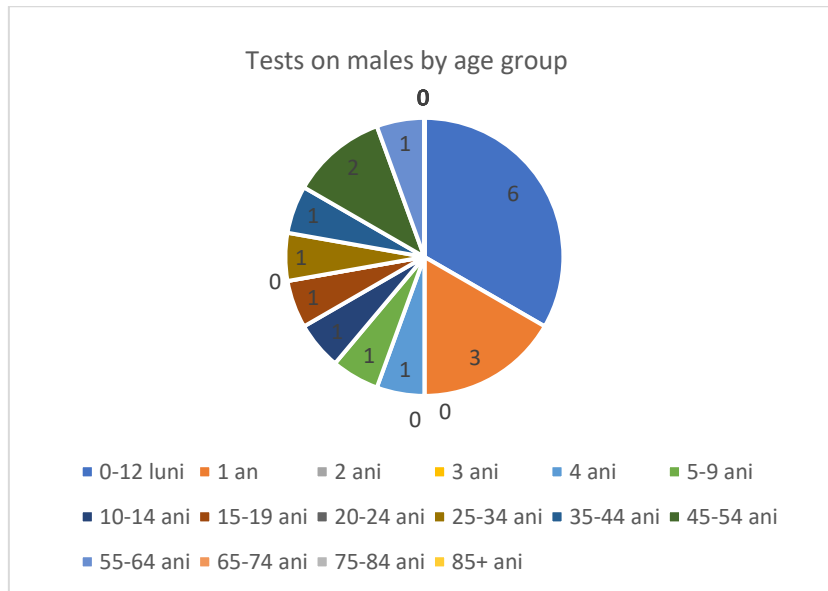


Figure 5 Tests on males by age group

Out of a total of 18 tests performed on males, 6 (33.33%) were performed in the age group 0-12 months, 3 (16.66%) were performed in the age

group 1 year, 2 (11.11%) were performed in the age group 45-54 years, and the remaining 7 (38.88%) were performed in other age groups (figure 5).

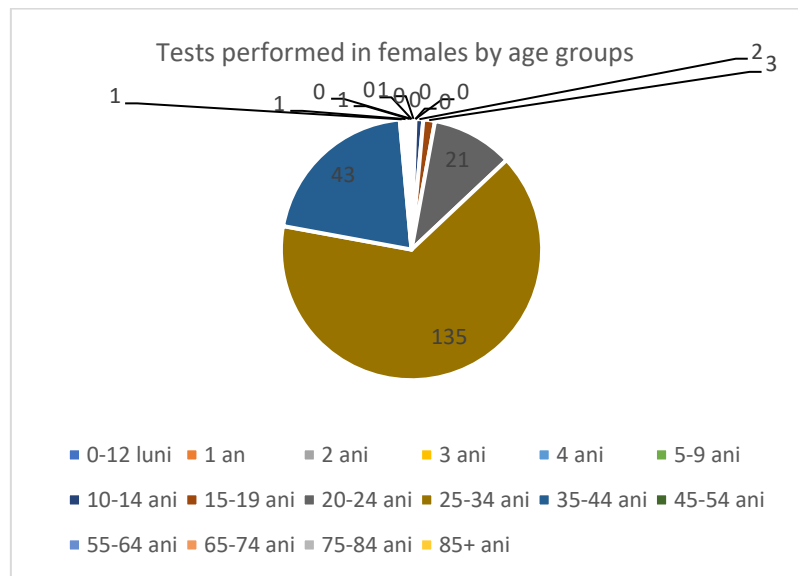


Figure 6 Tests performed on females by age group

Out of a total of 208 tests performed on females, 135 (64.90%) were performed in the age group 25-34 years, 43 (20.67%) were performed in the age group 35-44 years, 21 (10.09%) were performed in the age group 20-24 years, and the remaining 9 (4.32%) were performed in other age groups (figure 6).

Results of epidemiological investigations performed at the Praxis laboratory using the AB ANTI TOXOPLASMA GONDII- IgG (ELISA) test (table 2)

Table 2

Name Age group	Total	Total negative	Total positive	Positive Sex M	Positive Sex F	Total Sex M	Total Sex F
0-12 months	6	3	3	2	1	5	1
1 year	3	1	2	2	0	3	0
2 years	1	1	0	0	0	1	0
3 years	0	0	0	0	0	0	0
4 years	1	1	0	0	0	1	0
5-9 years	2	2	0	0	0	2	0
10-14 years	2	2	0	0	0	1	1
15-19 years	4	1	3	1	2	1	3
20-24 years	20	18	2	0	2	0	20
25-34 years	134	90	44	0	44	2	132
35-44 years	37	23	14	0	14	2	35
45-54 years	6	4	2	0	2	3	3
55-64 years	3	1	2	0	2	1	2
65-74 years	1	1	0	0	0	0	1
75-84 years	0	0	0	0	0	0	0
85+ years	0	0	0	0	0	0	0
TOTAL	220	148	72	5	67	22	198

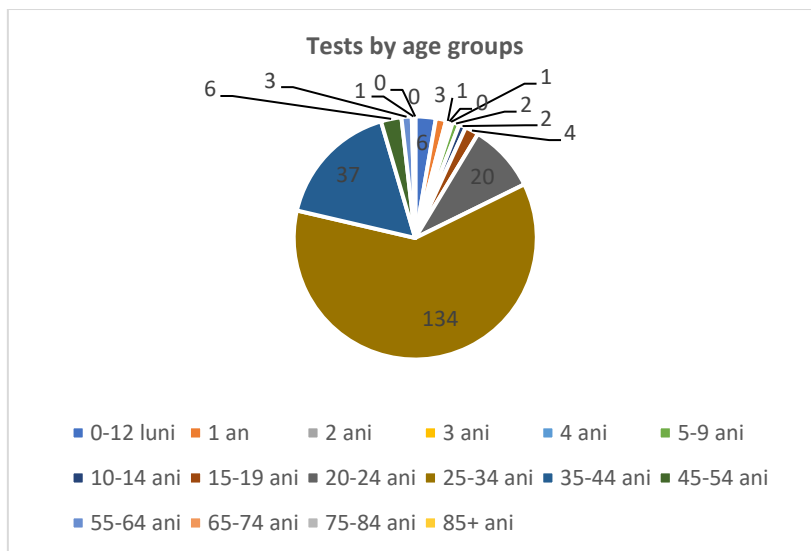


Figure 7 Tests by age groups

Out of a total of 220 tests performed, 134 (60.90%) were made in the age group 25-34 years, 37 (16.81%) were performed in the age group 35-44 years, 20 (9.09%) in the age group 20-24 years and the remaining 29 (13.18%) in

other age groups. The majority presence of IgG positive cases in the age group 25-34 years is also directly proportional to the high number of cases tested in this group, closely related to testing during pregnancy (figure 7).

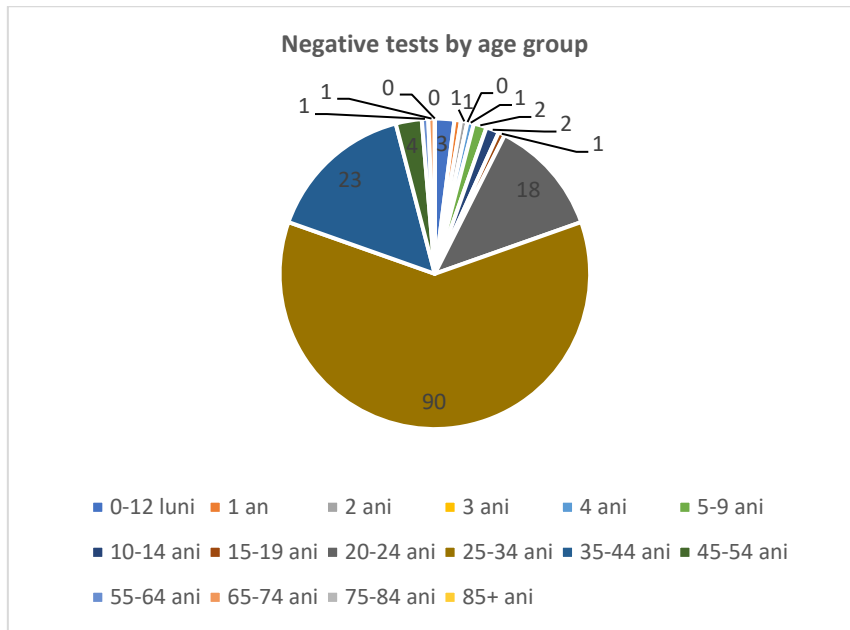


Figure 8 Negative tests by age group

Out of a total of 148 negative tests, 90 (60.81%) were performed in the age group 25-34 years, 23 (15.54%) in the age group 35-44 years, 18 (12.16%) in the age group 20-24 years and the remaining 17 (11.48%) in other age

groups. The high prevalence of negative cases in the same age group is influenced by the high number of tests for Toxoplasma at this age(*figure 8*).

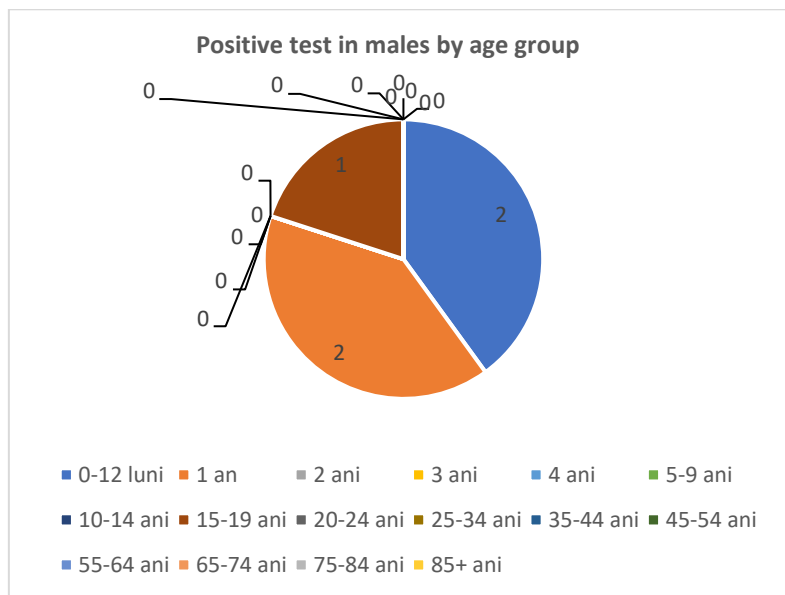


Figure 9 Positive tests in males by age group

Out of a total of 5 confirmed positive tests in males, 2 positive results were recorded in the age groups 0-12 months and 1 year, respectively, and 1 case was confirmed in the age group 15-19 years. Manifestation of the disease in children is related to a deficient

immune system under development or to transplacental transmission with manifestation after a variable period of time post birth (confirmed cases in the research literature(*figure 9*)).

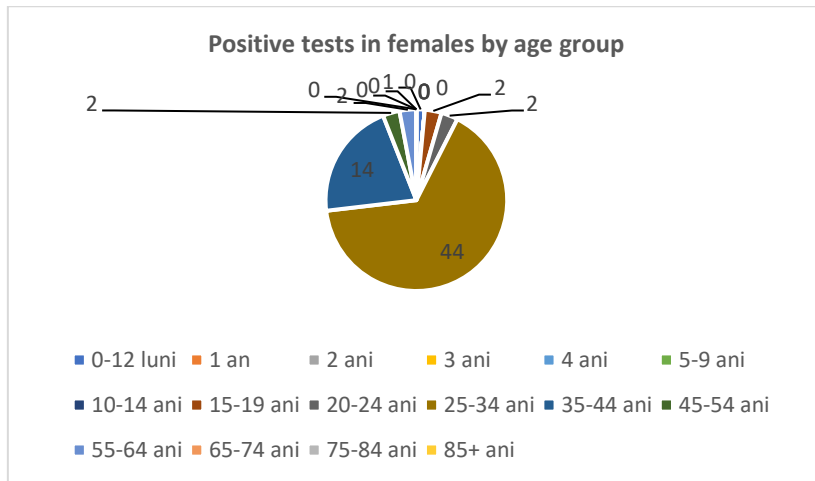


Figure 10 Positive tests in females by age group

Out of a total of 67 confirmed positive cases in females, 44 (65.67%) were in the age group 25-34 years, 14 (20.89%) in the age group 35-

44 years, and 9 cases were confirmed in other age groups (*figure 10*).

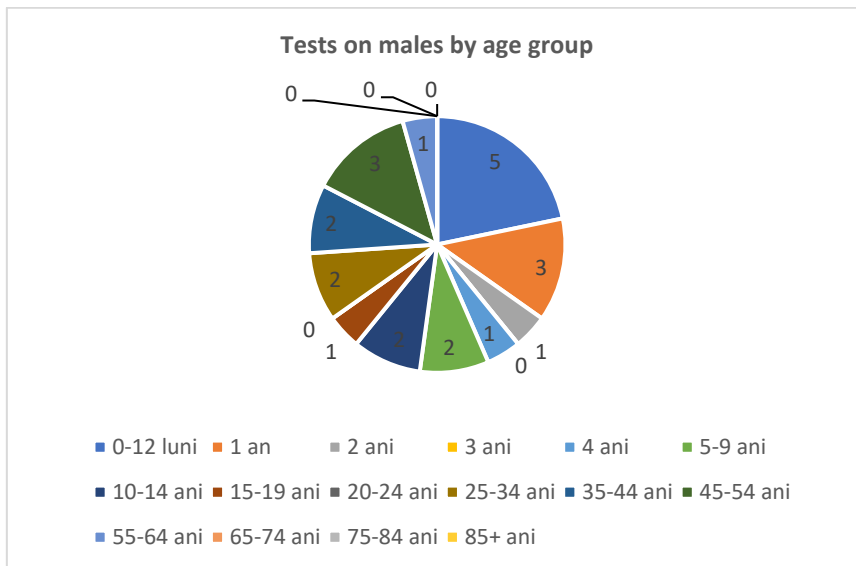


Figure 11 Tests on males by age group

Out of 22 tests performed in males, 5 were performed in the age group 0-12 months, 3 tests performed in the age groups 1 year and 45-54 years, 2 in the age groups 5-9 years, 25-34 years

and 35-44 years, and the remaining 5 were performed in other age groups (*figure 11*).

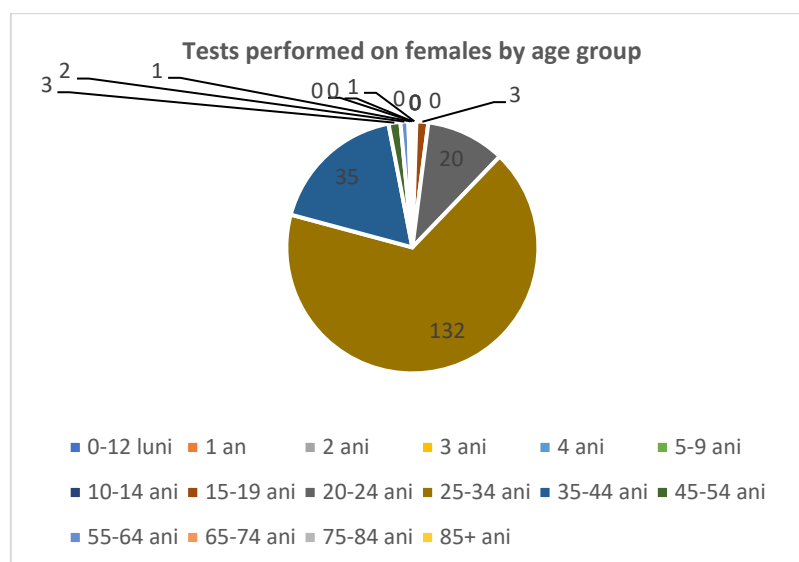


Figure 12 Tests performed on females by age group

Out of a total of 198 tests performed on females, 132 (66.66%) were performed in the age group 25-34 years, 35 (12.62%) in the age group 35-44 years, 20 (10.10%) in the age group 20-24 years and the remaining 11 were performed in other age groups (figure 12).

CONCLUSIONS

1. Studies on the spread of toxoplasmosis in animals and humans in Iasi County were carried out at the Faculty of Veterinary Medicine of "Ion Ionescu de la Brad" University of Life Sciences Iasi and at the Praxis medical tests laboratory.

2. Toxoplasmosis is an underdiagnosed protozoa in veterinary medicine due to the non-specific clinical picture, but is relatively well accounted for in human medicine due to the need for testing women during pregnancy. In cats the rapid test for antigen or Ig M is expensive to use in every clinical case suspected of a parasitosis, due to the non-specific clinical picture.

3. Toxoplasmosis is a very serious disease if contracted during pregnancy, causing miscarriage, severe malformations or nerve degeneration manifested even several years after birth. Also, in people with immunosuppressive diseases such as AIDS or autoimmune diseases, toxoplasmic encephalitis occurs, classifying toxoplasmosis as a very serious disease.

4. During 2020, 226 AB. ANTI TOXOPLASMA GONDII- IgM (ELISA) tests were performed in the Praxis laboratory, of which 15 were positive. All positive tests were identified only in females, of which 10 in the age group 25-34 years, 4 in the age group 35-44 years and 1 case in the age group 15-19 years. Most tests were requested by pregnant women during pregnancy.

5. In the Praxis laboratory during 2020, 220 more AB. ANTI TOXOPLASMA GONDII- IgG (ELISA) tests were performed, out of which 72 positive cases were identified, 5 being positive in males in the age groups 0-12 months, 1 year and 15-19 years, and the remaining 67 were identified in females in the following age groups: 0-12 months, 15-19 years, 20-24 years, 25-34 years, 35-44 years, 45-54 years and 55-64 years.

6. The majority of cases were recorded in women in the age group 25-34 years old, showing a much higher testing rate at this age, as this is a mandatory test during pregnancy. This shows that the protozoan *Toxoplasma gondi* is widespread in nature, as toxoplasmosis remains a neglected and under-diagnosed disease in Romania and can be an extremely serious disease.

7. The lack of positive cases in animals during the study, but their presence in large numbers in humans during a single year, shows the major importance of the study for public health, as this very serious disease in pregnant women is underdiagnosed in veterinary medicine.

8. The study is of major importance and warns us about the need to implement serological screening in animals in an attempt to eradicate this disease with serious repercussions for human health, especially in pregnant women and newborn babies.

9. Thus the results obtained can be used as material of high scientific value, in an attempt to educate the population on preventive measures against this disease, which can be contracted through the consumption of fruit, vegetables not properly washed, as well as through the consumption of undercooked meat, being a protozoan with a wide spread in nature.

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