

ISOLATION OF *TOXOPLASMA GONDII* FROM DOMESTIC AND WILD ANIMALS

Dimo Arnaudov, Atanas Arnaudov

*Faculty of Biology, University of Plovdiv,
„Tzar Assen“ 24, 4000 Plovdiv, Bulgaria
arny87@abv.bg*

ABSTRACT. 52 strains of *Toxoplasma gondii* with different virulence have been isolated from various domestic and wild animals. In most cases of isolation of toxoplasms (84.31%) it was about cystogenic strains, which are avirulent for the white mice. The virulent strains have been isolated mainly from hares. They caused acute toxoplasmosis among inoculated experimental animals (white mice, rabbits and pregnant ewes that had aborted).

Under epidemiological investigation it has been found that *Toxoplasma gondii* was a mean causing agent of abortions among ewes. The cats play important role in epidemiology of toxoplasmosis among the sheep. The wild animals perform an important role as a natural reservoir of *T. gondii* and a potential source of invasion with toxoplasms to the domestic animals and the man.

All isolated strains are immunologically identical in between them, as well as with the sample strain Rh.

KEY WORDS: *Toxoplasma gondii*, animals, epidemiology, micro precipitation, immunoelectrophoresis.

INTRODUCTION

Toxoplasmosis is a wide-spread zoonosis with natural focus, which affects a great number of domestic and wild animals, as well as humans. It is considered to be one of the main reasons for abortions and dead litters among animals. The isolation of strains of toxoplasms from animals and people is one of the most difficult moments related to the diagnosis of toxoplasmosis. The agent/cause for the disease *Toxoplasma gondii* was isolated and described for the first time in Tunis by Nicolle and Monceaux (1908) of the North African rodent *Ctenodactylus gondii*. In the same year Splendore had isolated toxoplasms from a rabbit in Brazil. A great number of strains of toxoplasms from different kinds of domestic, wild animals and humans had

been isolated in the following years. In our country Arnaudov (1973) for the first time isolated cistogenic strains of toxoplasms of rabbits and aborted fetus of goat.

Taking into consideration the actual state and practical significance of the matter „toxoplasmosis“, the objective of the present work shall be to present data from our long-year researches connected with the isolation of strains of toxoplasms from different types of domestic and wild animals. With this regard we made researches in the following directions:

- comparative researches on virulent and immunogenic features of the strains of toxoplasms isolated from animals
- establishing the importance of the strains of toxoplasms isolated by us in etiopathogenesis of abortions of sheep and goats.

MATERIAL AND METHODS

We carried out the isolation of strains of toxoplasms with bio-trials over white mice in the following two ways:

1. By intraperitoneal injection of suspension from internal organs in sterile physiological solution.
2. By digestive method of Jacobs et al. (1960).

We included in the experiment only animals seronegative for the toxoplasmosis–white mice, rabbits and ewes. After inoculating we observed the white mice every day within a period of 4-6 weeks. After that period we studied them serologically for toxoplasmosis, and the dead ones we examined by means of microscopes for ascertaining cysts in brain. The isolated strains of toxoplasms we maintained through periodic passages of white mice depending on the strain virulence (twice a week in virulent and 2-3 months for cystogenic ones). The immunological properties of the isolated strains we determined by comparing them with the sample strain Rh, by using the methods of microprecipitation in agar gel and immunoelectrophoretical analysis of serums from animals, experimentally infected with isolated strains (Friemel, 1984). The virulent properties of the strains we examined on white mice, pregnant ewes and domestic rabbits.

In two farms with large-scale of abortion on ewes we performed epidemiological investigation.

RESULTS AND DISCUSSIONS

The results of the bio-trials made are indicated in the Table. 52 strains of toxoplasms with different virulence have been isolated in Bulgaria for the first time – 22 from aborted fetuses of sheep; 3 – from aborted fetuses of goats; 1 – from the brain of an aborted swine; 14 from domestic rabbits; 7 – from wild rabbits; 4 – from minks; 1 – from a boar. We ascertained toxoplasms also in print specimens from the lien of a roe and 3 fecal specimens from domestic cats. The inoculated white mice reacted positively for toxoplasmosis as per microprecipitation reaction in agar gel after the 20th -28th day from their contraction. 1-3 precipitation lines formed between the antigen and blood serum of the white mouse. All animals from which we isolated toxoplasms reacted positively for microprecipitation reaction in the agar gel.

In a great percentage of the cases of isolation of toxoplasms – 43 (84.31%) it was about cystogenic strains (Fig. 1), which are avirulent for the white mouse. After 15 to 21 day of the inoculation in the brain of white mouse, cysts visible by means of a microscope with size 9,9 to 120 µm were ascertained. The cysts have a distinct round shape, it is more rare that they have an oval shape with a double membrane filled with bradyzoidts. The isolation of the virulent strains of toxoplasms had been realized even in the first passage, most frequently within 7-10 day after inoculation. Throughout that period the inoculated animals fell ill with acute toxoplasmosis (white mice, domestic rabbits and pregnant ewes that had aborted). The results of the comparative immunological researches of isolated strains of toxoplasms with the sample strain Rh are indicated on Fig. 2. It is evident that all strains are identical in between them, as well as with the sample strain. The results were similar also with the immunoelectrophoretic study of serums from sheep, experimentally invaded with toxoplasms (Fig. 3). Our researches carried out on a great number of domestic and wild animals indicated that toxoplasmosis has a wide-spread and general dissemination among some of them – sheep, rabbits and hares, goats, minks, boars. All this proves undoubtedly that wild animals play a significant role in maintaining the natural focus of toxoplasmosis.

Under epidemiological investigation in farms with large-scale abortion on ewes we found the following:

1. *Toxoplasma gondii* was a mean causing agent of these abortions.
2. The ewes and the forage were in direct contact with wandering cats. This fact shows us the important role of the cats in epidemiology of toxoplasmosis among the sheep.

It impresses that in comparison with other authors (Rašin, 1970) all strains in our researches isolated from hares are virulent. This proves that *T. gondi* plays a significant role in the pathology of hare. Additional evidence is the isolation of toxoplasms from dead animals found by accident. The data from our researches confirm Buxton's theory (1986) (for causing abortions of pregnant sheep), that death of fetuses is caused by their mutations occurring in brain and anorexia caused by the changes in cotyledons of the placenta.

CONCLUSIONS

1. It has been isolated for the first time in Bulgaria 52 strains *Toxoplasma gondii* with different virulence from domestic and wild animals – 8 virulent and 44 cystogenic.
2. The isolated strains are abortogenic for pregnant ewes and goats.
3. The strains are identical immunologically.
4. The wild animals perform an important role as a natural reservoir of *Toxoplasma gondii* and a potential source of invasion with toxoplasms to the domestic animals and the man.

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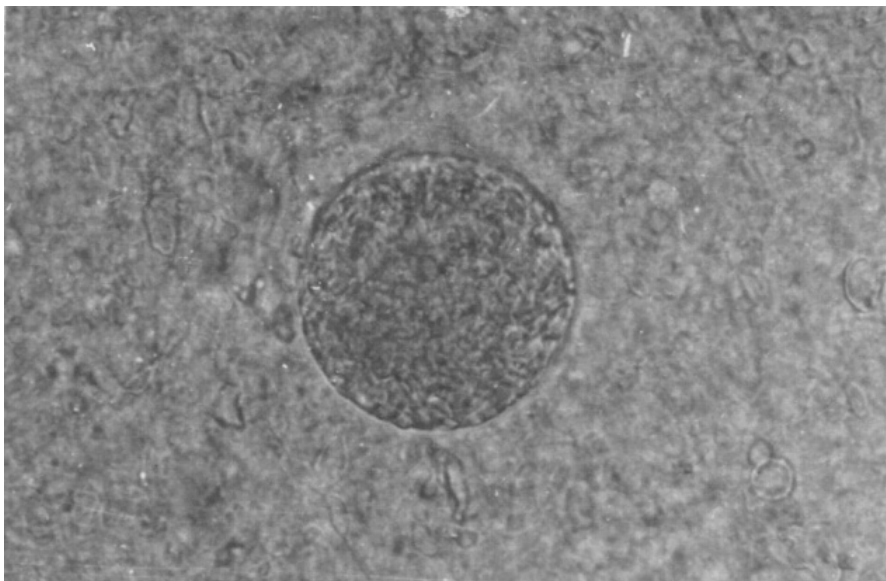
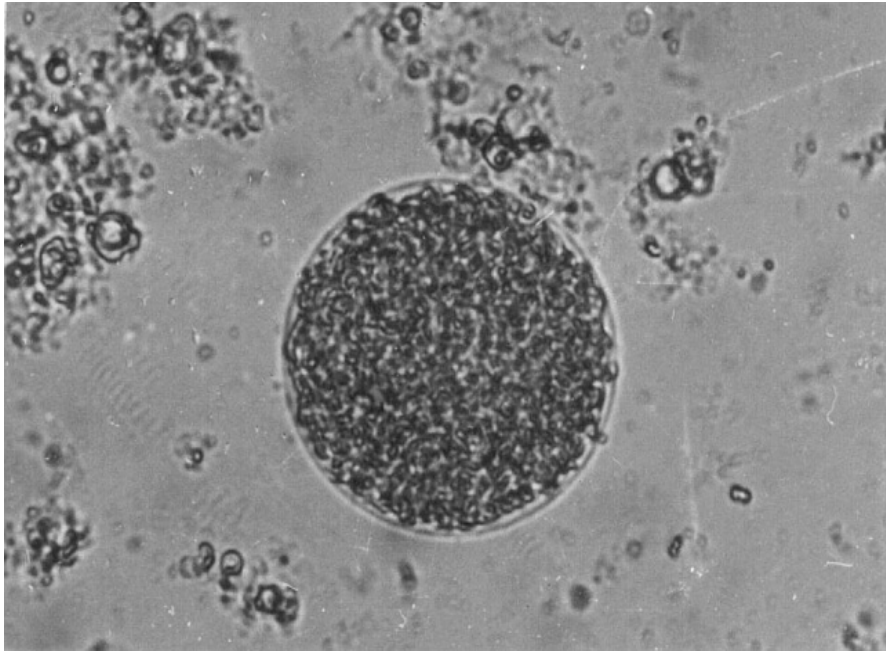


Fig.1. *Cystogenic strains Toxoplasma. gondii*

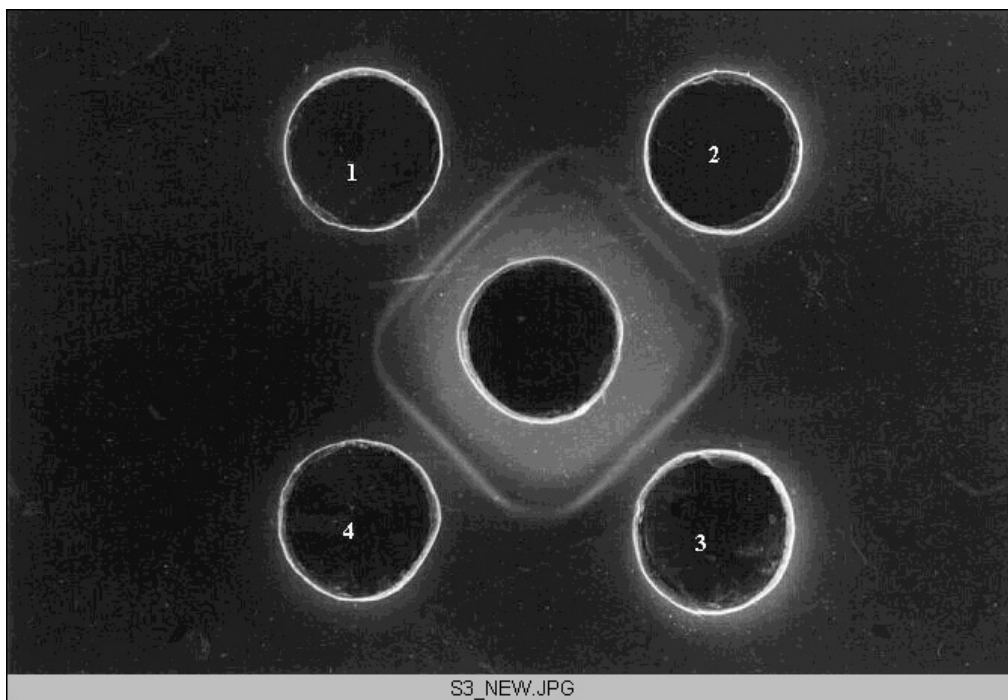


Fig.2 Micro precipitation in agar gel. Full merging of the precipitation lines.

Central pit –antigen received from trophozooids of high virulent strain Rh.

Peripheral pits- 1- rabbit antiseum to sample strain Rh; 2, 4- rabbit antiseum to cystogenic strain T. gondii, isolated from rabbit; 3- rabbit antiseum to cystogenic strain T. gondii, isolated from sheep's fetus.

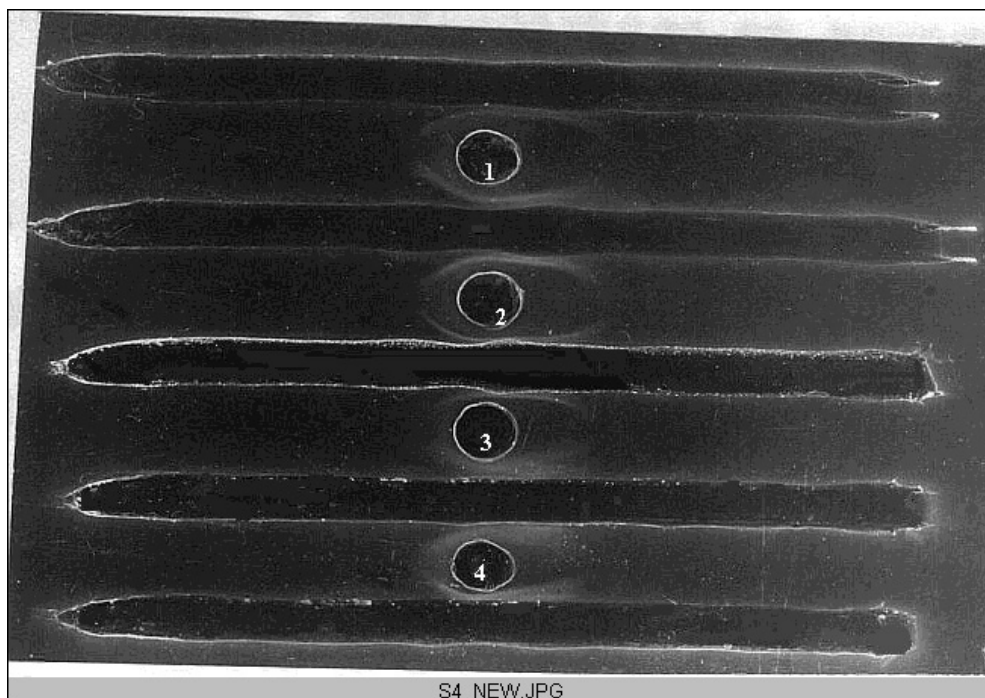


Fig.3 Immunoelectrophoretic analysis of serums from ewes experimentally infected with various strains T. gondii. Immunological identity of antiserums to cystogenic strains and sample strain Rh.

Round pits: 1, 3, 4- antiserums to cystogenic strains; 2- antiserum to sample strain Rh.

Long pits - sample strain Rh.

Table 1. Isolation of *Toxoplasma gondii* from domestic and wild animals

Animals	Samples		Methods		Isolated strains		
	total number	from fetuses	Digestive	Rutine	total number	Cystogenic	<i>Virulent.</i>
Sheep	203	44	159	44	22	22	-
Goats	7	7	-	-	3	3	-
Cattle	20	-	20	-	-	-	-
Pigs	230	19	211	19	1	1	-
Rabbits	20	-	-	20	14	14	-
Minks	11	-	-	11	4	4	-
Wild boars	12	-	-	12	1	-	1
Roes	3	-	-	3	-	-	-
Deer	1	-	-	1	-	-	-
Pheasants	6	-	-	6	-	-	-
Hares	62	-	-	62	7	-	7
Wood-grouses	2	-	-	2	-	-	-
Moufflons	1	-	-	1	-	-	-
Martens	1	-	-	1	-	-	-
Total	579	70	390	189	52	44	8