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TOXOPLASMOSIS IN ZOO ANIMALS

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Abstract. Blood samples (collected and dried on filter paper) from 2 338 animals which had died in the zoological gardens in Berlin and other cities in the German Democratic Republic were examined by indirect haemagglutination test. Toxoplasma antibodies were found in 430 (18.3%) animals. Out of this number 1 113 were mammals and among them 201 (18.1%) were found to be positive. Out of 1 225 birds 228 (18.6%) were positive. A total of 194 species or subspecies (89 mammal species and 105 bird species) reacted positively. On the basis of these findings the spread of toxoplasma infection under zoo conditions as an artificial ecosystem in urban environment is discussed.

A zoological garden, particularly if located in the territory of a large city, represents an artificial ecosystem with a wide spectrum of exotic animal species living in conditions dissimilar to those in natural biotopes. This ecosystem is penetrated by synanthropic or domestic animals from the surrounding urban and suburban environment: birds, mainly sparrows, pigeons and doves, mouse-like rodents, stray dogs and homeless cats. Even zoo visitors get rid of undesirable kittens here (Prof. Dathe, pers. com.). Toxoplasma infection may be brought in the zoo area primarily by fresh animals imported from their native country; this fact may be anticipated due to the cosmopolitan prevalence of this disease. Stress condition after transport or concurrent infection or other causes promote the activation of toxoplasmosis manifesting itself as a fatal disease. Secondarily, the animals may become infected by alimentary route. Carnivorous animals (predators) become infected with zoitocysts in animal food. The infection risk is higher here than in nature due to the fact that carnivores are fed raw meat coming from the domestic animals which are more often exposed to the infection under conditions of cultivated landscape than wild animals which constitute the natural food of exotic flesh-eaters in their initial biotopes. Herbivorous, granivorous and omnivorous animals become infected with oocysts in contaminated food or soil into which the oocysts are shed with the faeces of cats. After Hilgenfeld (1965) important is also the fact that different animal species with different degree of susceptibility are concentrated in the zoo facilities. Repeated infections were described after placing new animals in cages where previous fatal cases of toxoplasmosis had occurred.

The fact that some disease cases in zoos may be zoonoses (Rosický 1978 a, b), potentially hazardous to the zoo attendants (Hilgenfeld 1963), visitors or human inhabitants of the surrounding urban environment, is not negligible either.

In previous papers dealing with toxoplasmosis incidence in exotic animals only single cases were described. We are citing here studies where greater attention has been paid to the problem.

Ratcliffe and Worth (1951) identified toxoplasmosis in 27 animals, namely in 13 mammals (10 species) and 14 birds (5 species) in the Philadelphia zoo. The disease was diagnosed on the basis of a histological examination. The course of illness was mostly acute. Toxoplasma etiology was revealed in less than 1% of animals which had died in a decade. Hilgenfeld (1965) verified the toxoplasma presence in 25 kangaroos by histological examination at necropsies conducted at the Berlin Zoo, namely in 4 *Wallabia rufogrisea* and in 16 *Macropus rufus*. The animals died in the period of six years. The author incriminates toxoplasmosis particularly for the death of kangaroos. From

the epizootiological aspect important are the findings in rodents trapped in the zoo runs. Toxoplasmas were found in two out of 25 Norway rats and in three out of 8 mice. Mandelli et al. (1966) described three fatal cases in a colony of *Wallabia rufogrisea frutica* in the Milan Zoo. Toxoplasmosis was verified by isolation test. Pathological material was abounding in evidence of disseminated encephalitis, acute inflammatory and ulcerous gastritis, enteritis and focal myocarditis. Poelma and Zwart (1972) described a toxoplasmosis epizootic in 13 birds (Columbiformes) in the Rotterdam Zoo. The infection was identified microscopically and by isolation tests. Riemann et al. (1974) investigated by microtitre system of indirect haemagglutination test 109 blood samples from exotic animals, obtained from a veterinary clinic, zoo or from private animal breeders. The highest prevalence of antibodies was revealed in Felidae (60%), Marsupialia (54%) and Canidae (50%). High titres were detected in *Zalophus californianus* and *Lama guanicoe lama* (*L. peruana*), in *Macropus robustus*, in two *Profelis temmincki*, in two *Otocolobus manul* and in *Elephas maximus*. The authors also used the complement-staining fluorescent-antibody test which well agreed with haemagglutination test. Schröder and Ippen (1976) found toxoplasmosis to be the death cause of 20 (17.1%) out of 117 dead kangaroos dissected at necropsies in the Berlin Zoo. They were 15 *Macropus rufus*, three *Wallabia rufogrisea* and two *Thylogale eugenii*.

Due to the occurrence of fatal cases of toxoplasmosis in the Berlin Zoo we started in 1973 our studies on the spread of this disease under zoo conditions. In the first stage of our research we traced the prevalence of toxoplasma antibodies in the necropsied animals.

MATERIAL AND METHODS

The blood to be examined was collected from the dissected heart or from the pleural cavity and smeared on filter paper (Schleicher et Schuell No. 589[3]). After soaking and drying up the blood samples were put in paper envelopes, wrapped in plastic and sent to laboratory. Prior to examination the material was stored in refrigerator. Blood stains were cut out from the paper in the shape of a disc 25 mm in diameter and eluted in 0.2 ml of phosphate saline solution pH 7.2 in a plastic tube. The method used was actually the method of Anderson et al. (1961) for the detection of schistosomiasis, introduced by Kramář (1965) for the detection of toxoplasma antibodies using indirect fluorescent antibody test. Later on elution technique was used in a glass tube.

Indirect haemagglutination test (IHT) with antigen prepared from mouse peritoneal exudate was employed. In order to demonstrate the toxoplasma antibodies lyophilized sheep erythrocytes fixed in formalin and tanin (commercial preparation SEVATEST TK-HEM) were used as antigen carrier. The reliability of our IHT was verified while investigating both human (Kozojed et al. 1971, 1980) and animal sera (Arnaudov et al. 1976, Kozojed et al. 1975).

RESULTS

Blood samples of 2 338 animals were examined. *Toxoplasma* antibodies were detected in 430 (18.3%) animals. Out of this number 1 113 were mammals (Table 1), among them 201 (18.1%) were positive. Out of 1 225 birds (Table 2) 228 (18.6%) were positive. A total of 194 species or subspecies, namely 89 mammal species and 105 bird species were positive.

From the Berlin Zoo 749 mammals were investigated, 140 (18.7%) being positive. Out of 777 birds 167 (21.5%) were positive. A total of 1 526 animals were investigated.

Apart from the Berlin Zoo the animals were provided from the zoos in Magdeburg, Dresden, Cottbus, Erfurt, Eberswalde, Forst, in single cases also from private animal breeders in the German Democratic Republic and from the zoo in Wroclaw (Poland), where 364 mammals were investigated, 61 (16.8%) being positive. Out of 448 birds investigated 71 (15.8%) were positive.

In the text below we are listing systematic groups of mammals (species or subspecies) in which positive cases were detected. In some cases the German animal name stated in the necropsy protocole was insufficient and the identification of the animal could be

Table 1. Results of serological examination for toxoplasmosis (indirect haemagglutination test, dried blood on filter paper) in 1113 mammals which had died in zoological gardens in Berlin and other places of German Democratic Republic (1973—1978)

Zoo	Macropodidae			Simiae			Carnivora		
	Examined	Positive	percent	Examined	Positive	percent	Examined	Positive	percent
Berlin	27	4	14.8	17	3	17.6	83	8	9.6
Others	22	1	4.5	50	5	10.0	30	6	20.0
Total	49	5	10.2	67	8	11.9	113	14	12.4
Zoo	Felidae			Rodentia et alia			Perissodactyla		
	Examined	Positive	percent	Examined	Positive	percent	Examined	Positive	percent
Berlin	84	7	8.3	44	8	18.2	41	6	14.6
Others	39	6	15.4	23	5	21.7	20	4	20.0
Total	123	13	10.6	67	13	19.4	61	10	16.4
Zoo	Artiodactyla			Cervidae			Caprinae		
	Examined	Positive	percent	Examined	Positive	percent	Examined	Positive	percent
Berlin	176	35	19.9	147	36	24.5	130	33	25.4
Others	66	15	22.7	53	9	17.0	61	10	16.4
Total	242	50	20.7	200	45	22.5	191	43	22.5

done only within genus, family or order. The birds are listed under generic names only, the positive species being pointed out.

The following mammal groups were investigated:

Out of 49 representatives of the family Macropodidae antibodies were detected in 5 (10.2%) animals. The following 3 species were positive: *Wallabia agilis*, *Macropus* sp. and *Dendrolagus* sp.

Out of 67 representatives of the suborder Simiae (Anthropoidea) antibodies were detected in 8 (11.9%) animals. The following 8 species were positive: *Alouatta* sp., *Saimiri sciureus*, *Cercopithecus* sp., *Colobus* sp., *Macaca mulatta*, *Cynopithecus maurus*, *Papio hamadryas* and *Gorilla gorilla*.

Out of 113 representatives of the order Carnivora (except Felidae) antibodies were found in 14 (12.4%) animals. The following 12 species were positive: *Procyon* sp. (Procyonidae); *Ailurus fulgens* (Ailuridae); *Thalarctos maritimus*, *Helarctos malayanus* and *Tremarctos ornatus* (Ursidae); *Genetta* sp. and *Paradoxurus* sp. (Viverridae); *Canis dingo*, *C. lupus*, *Alopex corsac*, *Fennecus zerda* and *Chrysocyon brachyurus* (Canidae).

Out of 123 representatives of the family Felidae antibodies were detected in 14 (10.6%) animals. The following 9 species were positive: *Felis catus*, *F. silvestris*, *F. chaus*, *Prionailurus bengalensis*, *Lynx lynx canadensis*, *Puma concolor*, *Panthera pardus*, *P. tigris* and *P. leo*.

In the group including 67 representatives of the order Rodentia and other mammal orders antibodies were detected in 13 (25.0%) animals. The following 10 species and subspecies were positive. *Castor fiber*, *Mesocricetus auratus*, *Cricetulus* sp., *Jaculus jaculus*, *Hystrix* sp., *Cavia* sp., *Octodon degu*, *Oryctolagus* sp., *Erinaceus* sp., *Dasypus* sp.

Out of 61 representatives of the order Perissodactyla antibodies were detected in 10 (16.4%) animals. The following 5 species and subspecies were positive: *Equus caballus* (Shetland pony), *E. asinus* (various breeds), *Equus burchelli chapmanni*, *E.b. antiquorum* (Equidae), *Tapirus* sp. (Tapiridae).

Out of 242 representatives of the order Artiodactyla (except Cervidae and Caprinae) antibodies were detected in 50 (20.7%) animals. The following 23 species and subspecies were found to be positive: *Sus scrofa*, *Sus domesticus* of the Chinese breed (Suidae); *Camelus dromedarius*, *C. ferus*, *Lama guanicoe*, *L. guanicoe pacos* and *L. vicugna* (Camelidae); *Giraffa camelopardalis* (Giraffidae); *Taurotragus oryx*, *Boselaphus tragocamelus*, *Tetracerus quadricornis*, *Addax nasomaculatus*, *Damaliscus d. dorcas*, *Connochaetes gnou*, *C. taurinus*, *Antilope cervicapra*, *Saiga tatarica*, *Ovibos moschatus*, *Bos primigenius taurus*, *B. primigenius indicus*, *B. javanicus*, *B. gaurus* and *Bison bonasus* (Bovidae).

Out of 200 representatives of the family Cervidae antibodies were detected in 45 (22.5%) animals. The following 14 species and subspecies were found to be positive: *Moschus moschiferus*, *Muntiacus muntjak*, *Odocoileus virginianus*, *O. hemionus*, *Rangifer tarandus*, *Elaphurus davidianus*, *Axis axis*, *Rusa* sp., *Dama dama*, *Cervus nippon*, *C.n. pseudaxis*, *C. elaphus*, *C.e. maral*, *C.e. bactrianus*.

Out of 191 representatives of the subfamily Caprinae antibodies were found in 43 (22.5 %) animals. The following 5 species were positive: various breeds of *Capra hircus* (Cameroon goat), *Hemitragus jemlahicus*, *Ovis orientalis*, *O. musimon* and different breeds of *O. aries* ("Zackelschaf", Astrakhan sheep etc.).

The following bird groups were investigated: Out of 85 representatives of the order Ciconiiformes antibodies were detected in 14 (16.5 %) cases. A total of 8 species belonging to the following genera was found to be positive: *Ardeola*, *Ciconia* (2 species), *Egretta*, *Platalea*, *Plegadis*, *Pseudotantalus*, *Tigrisoma*.

Out of 341 representatives of the order Anseriformes antibodies were detected in 88 (25.8 %) cases. A total of 35 species belonging to the following genera was positive:

Table. 2. Results of serological examination for toxoplasmosis (indirect haemagglutination test, dried blood on filter paper) in 1225 birds which had died in zoological gardens in Berlin and other places of German Democratic Republic (1973—1978)

Zoo	Ciconiiformes			Anseriformes		
	Examined	Positive	percent	Examined	Positive	percent
Berlin	54	9	16.7	256	73	28.5
Others	31	5	16.1	85	15	17.6
Total	85	14	16.5	341	88	25.8

Zoo	Galliformes			Ralliformes		
	Examined	Positive	percent	Examined	Positive	percent
Berlin	65	13	20.0	64	9	14.1
Others	26	4	15.4	15	1	6.7
Total	91	17	18.7	79	10	12.6

Zoo	Columbiformes			Psittaciformes		
	Examined	Positive	percent	Examined	Positive	percent
Berlin	54	22	40.7	52	11	21.2
Others	14	2	14.3	127	24	18.9
Total	68	24	35.3	179	35	19.6

Zoo	Passeriformes			Alia		
	Examined	Positive	percent	Examined	Positive	percent
Berlin	28	2	7.1	204	18	8.8
Others	48	10	20.8	102	10	9.8
Total	76	12	15.8	306	28	9.2

Aix (2 species), *Amazonetta*, *Anas* (7 species), *Anser* (4 species), *Aythia* (4 species), *Branta* (2 species), *Bucephala*, *Casarca*, *Cereopsis*, *Cygnus* (2 species), *Dendrocygna* (2 species), *Mergus* (2 species), *Netta* (2 species), *Oxyura*, *Somateria*, *Tadorna* and *Tychyeres*.

Out of 91 representatives of the order Galliformes antibodies were detected in 17 (18.7 %) cases. A total of 10 species belonging to the following genera was positive: *Alectoris*, *Argusianus*, *Colinus*, *Coturnix*, *Crossoptilon*, *Lophortyx*, *Numida*, *Pavo*, *Phasianus* and *Syrmaticus*.

Out of 79 representatives of the order Ralliformes antibodies were found in 10

(12.6 %) cases. Three species belonging to the following genera were positive: *Anthropoides*, *Grus* and *Otis*.

Out of 68 representatives of the order Columbiformes antibodies were detected in 24 (35.3 %) cases. The following genera were found to be positive: *Columba* (2 species), *Goura*, *Zenaida*, and an unspecified representative of the subfamily Treroninae. A total of 5 species was positive.

Out of 179 representatives of the order Psittaciformes antibodies were detected in 35 (19.6 %) cases. A total of 16 species belonging to the following genera was positive: *Agapornis*, *Amazona*, *Ara*, an unspecified representative of the subfamily Cacatuinae, *Cyanoramphus* (2 species), *Lorius* (2 species), *Neophrena*, *Melopsittacus*, *Nestor*, *Nymphicus*, *Platycercus* (3 species) and *Psittacus*.

Out of 76 representatives of the order Passeriformes antibodies were detected in 12 (15.8 %) cases. A total of 10 species belonging to the following genera was positive: *Acridotheres*, *Amandina*, *Copsychus*, *Gracula*, *Erithacus*, *Estrilda* (2 species), *Fringilla*, *Monticola* and *Ortygospiza*.

In a mixed group of other bird orders antibodies were detected in 28 (9.2 %) cases out of 306 specimens examined. A total of 18 species belonging to the following orders was found to be positive: Struthioniformes with a representative of the genus *Rhea*, Sphenisciformes with a representative indicated as "pinguin", Podicipediformes with a representative of the genus *Podiceps*, Pelecaniformes with representatives of the genera *Pelecanus* and *Phalacrocorax*, Phoenicopteriformes with representatives of the genera *Phoenicopterus* and *Phoeniconaias*, Falconiformes with representatives of the genera *Haliaeetus*, *Falco* (2 species) and with a representative of the subfamily Aegypiinae, Tinamiformes with an unspecified representative, Strigiformes with representatives of the genera *Bubo* and *Asio*, Cuculiformes with representatives of the genera *Cuculus* and *Centropus*, Coraciadiformes with a representative of the genus *Coracias*, and Piciformes with a representative of the genus *Rhamphastus*.

DISCUSSION

As far as feral hosts of *Toxoplasma gondii* are concerned the zoological garden holds a special position. The prevalence of toxoplasmosis is higher here than in free nature, but it does not reach the level usual in the farms of some domestic animals, notably pigs. Our study demonstrates a wide host spectrum of toxoplasmosis. In our material the incidence of haemagglutination antibodies was 18 % on the average. Riemann et al. (1974) found the antibody prevalence in zoos to be higher (32 %), emphasizing the favourable conditions for the spread of infection due to the vicinity of Felidae which are fed raw meat and freshly killed birds. Dobos—Kovács et al. (1974) recognized the connection between the deaths of kangaroos in the Budapest Zoo and the oocysts found both in the dry and fresh excrements of cats in the food store-rooms.

In this connection we point out the potential source of infection in birds of the order Columbiformes. In this group the highest prevalence of antibodies (40.7 %) was found in Berlin, the majority of the birds being feral pigeons (16 out of 22) visiting the zoo area from the adjoining urban environment and shot here. In the Prague Zoo the pigeons and doves are used as animal food (Veselovský, personal communication). Important are the finds of Čatár (1974) who made two positive isolations from 16 examined specimens of *Columba livia* obtained directly from the urban area of Bratislava, and six positive isolations from 12 examined specimens of *Streptopelia decaocto*. The author considers the pigeons to be potential reservoirs of toxoplasmosis in urban conditions.

The desirable objective of further research will be the prevention of toxoplasmosis under zoo conditions, which would help forestall epizootics and losses of rare animals.

ТОКСОПЛАЗМОЗ У ЖИВОТНЫХ В ЗООПАРКАХ

Р. Иппен, В. Козоед и Й. Йира

Резюме. Засохшие на фильтровальной бумаге образцы крови, взятые у 2338 животных, погибших в зоопарках в Берлине и других городах Германской Демократической Республики, исследованы с помощью непрямой реакции гемаглютинации. Антитела против возбудителя токсоплазмоза обнаружены у 430 (18.3 %) животных. Из этого числа 1113 были млекопитающие и среди них 201 (18.1 %) оказались положительными. Из числа 1225 птиц 228 (18.6%) были положительны. Всего 194 видов и подвидов (89 видов млекопитающих и 105 видов птиц) имели положительную реакцию. На основании этих находок в работе рассматривается распространение токсоплазмоза в условиях зоопарка как искусственной экосистемы городской окружающей среды.

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