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Domestic ducks are often confined on natural bodies of water which are also inhabited by wild waterfowl, so that the possibility exists for the two to exchange parasitic protozoa, in particular, trichomonads.

V. L. Yakimov (1931), F. Doflein, E. Reichenow (1953), C. M. Wenyon (1926), P. Grassi (1952), B. A. Timofeyev, and V. V. Petrovsky (1967) have named 27 species of trichomonads of birds, found in domestic chickens, ducks, geese, pigeons, guinea-fowl, turkeys, pheasants, hazel-grouse, owls, quail, partridges, cuckoos, African cormorants, the American coot (Fulica americana) and several other species of birds.

Hitherto no one has studied the intestinal trichomonads of wild waterfowl in Kazakhstan. In the period from March thru October, 1962, working in conjunction with the helminthologic and ornithologic divisions of the Institute of Zoology of the Academy of Sciences, Kazakh SSR, along the lower Asy River, on Lake Biylikul', Dzhambul Oblast' and on Lake Akzhar in the Chimkent Oblast', we had the opportunity of studying several wild waterfowl suspected of carrying intestinal trichomonads. The birds used in the research were caught by associates of the Laboratory of Birds, Reptiles and Amphibians and the Helminthology Laboratory of the Institute of Zoology, Academy of Sciences of the Kazakh SSR. (D. I. Chekmenev, E. F. Rodionov, B. C. Korobkin. M. P. Shakhvorostov also took part in the work.)

Portions of the birds studied included contents and scrapings from the mucosa of the large intestine, both fresh preparations with physiologic solutions and in smears stained according to the Romanovsky method.

In all, 381 birds, of 19 species, were studied, of which 37.7% were infected with trichomonads (see table). Extent of invasion was high in Netta rufina Pall., Nyroca ferina L., the pintail (Anas acuta L.), gray ducks, gray geese and Gallinula chloropus L. Trichomonads were not observed in Anas clypeata L., common gulls, Chlidonias nigra L., despite the fact that 67 of these birds were dissected. In addition, we did not find them in Nyroca nyroca Guld., Podiceps caspicus Hall., Ardea alba L., Ardea cinerea L., Tadorna tadorna L., Oxyura leucocephala Scop. and Phalacrocorax carbo L., apparently because these birds were not studied very much.

Intensity of infection was strong only in 2 Netta rufina, 3 Anas crecca L. and 5 Fulica atra L., in which we found up to 40 trichomonads per microscope field at a magnification of 600X. The parasites we isolated from the large intestine of several species of waterfowl were structurally similar to Trichomonas anatis from the cecum of domestic ducks. Thus, most of the trichomonads described below we assign to T. anatis (Kotlan, 1923), except for the trichomonads of the geese and Fulica atra.

The trichomonads which we isolated from the various species of birds have the following structure:

Trichomonads of the pintail (Anas acuta)--Fig. 1, 1-4. In fresh preparations most of the trichomonads are ovoid with a sharpened posterior end, but in place of this we sometimes encountered bacilloid, bean-shaped and round forms. In stained smears the cytoplasm is sharply divided between ecto- and endoplasm. At the anterior pole one can see, weakly separated from the ectoplasm, violet granules or blepharoplasts, from which arise the 4 anterior flagella of unequal length and one posterior flagellum, which borders on the undulating membrane. The anterior flagella are approximately as long as the body or slightly shorter. The free part of the posterior flagellum is approximately as long as the anterior flagella. Trichomonads with 2 or 3 anterior flagella also occur. The nucleus is compact, dark violet, oblong or angular; it is close to the blepharoplast and shields the anterior end of the axostyle. The axostyle is broad (up to 2 μm), weakly delineated from the cytoplasm, stained evenly and protrudes up to 5 μm from the posterior end of the body in the form of a sharp spike. The costa is well developed and situated directly at the base of the undulating membrane in the form of a broad violet stripe. The undulating membrane is well developed. The trichomonads are 6.4-13.6 μm long and 4.2-8.0 μm wide.

Trichomonads of gray ducks (Anas strepera)--Fig. 1, 5-7. In fresh preparations these trichomonads are spindle-shaped or ovoid, with pointed anterior and posterior ends. We also encountered bacilloid forms with a pointed posterior end. In stained smears the ecto- and endoplasm are not separated. The cytoplasm is granular, with brightly-colored vacuoles. Violet granules (blepharoplasts) are easily seen at the anterior end. From them come the 4 anterior flagella in pairs, their lengths unequal, and one posterior flagellum bordering the undulating membrane. In most trichomonads the length of the anterior flagella is equal to the body length or somewhat shorter. The length of the free portion of the posterior flagellum is less than that of the anterior. Trichomonads with 2 and 3 anterior flagella occur. The nucleus is compact, often spindle-shaped or ovoid, with dark aggregations of chromatin; it is situated obliquely and dorsoventrally behind the blepharoplast. In some individuals the anterior end of the nucleus touches the blepharoplast, shielding the anterior end of the axostyle. The axostyle is violet, filament-like, easily visible in the cytoplasm and protrudes markedly at the posterior end of the body (up to 6 μ) in the form of a filament-like outgrowth, but thicker than the posterior flagellum. A delicate costa is situated closer to the central longitudinal axis of the trichomonad and somewhat distant from the base of the undulating membrane. The undulating membrane is broad (up to 3 μ) and well developed. The trichomonads are 6.3-15.8 microns long and 4.3-9.0 microns wide.

Trichomonads of the common teal (Anas crecca)--fig. 1, 8-10. In fresh preparations these trichomonads have an oval form. They are also bacilloid, with a pointed posterior end, bean-shaped, half-moons and spheres. In stained smears the ecto- and endoplasm are not noticeably differentiated. The cytoplasm stains lightly and contains bright vacuoles and tiny basophilic granules. Visible at the anterior end are violet granules (blepharoplasts) from which the 4 anterior flagella of varying length arise in pairs and the one posterior flagellum, which borders the undulating membrane that ends up unattached at the posterior end of the body. The anterior flagella in most trichomonads are somewhat shorter than the body length. The free part of the posterior flagellum is somewhat shorter than the anterior ones. Trichomonads with 2 anterior flagella occur. The nucleus is compact, with aggregations of chromatin, most often rounded or ovoid, and lies somewhat distant from the blepharoplast. The filament-like axostyle is hard to see inside the cytoplasm in most cases and emerges at the posterior end of the body in the form of a sharp spike (up to 4 μ long). The costa is well developed and passes immediately along the base of the well developed undulating membrane.

Body length is 6.4-15.6 microns; width is 4.5-9.0 microns.

Trichomonads of the mallard (Anas platyrhynchos)--fig. 1, 11-13. In fresh preparations these trichomonads have an oval form. Pearshaped and round individuals occur. In stained smears the cytoplasm colors evenly and cannot be separated into ecto- and endoplasm. However, the dorsal surface and the base of the undulating membrane stain more heavily. The cytoplasm is granular and contains small basophilic granules. Violet granules (blepharoplasts) on the rounded anterior end are visible. From them the 4 anterior flagella arise in pairs of different lengths. A posterior one bordering the undulating membrane also arises from them. In most trichomonads the anterior flagella and the free part of the posterior flagellum are half the body length. The nucleus is compact with aggregations of chromatin. It is flask-shaped more often than not, and lies immediately against the blepharoplast. A filament-like axostyle is prominently visible in the cytoplasm in most individuals, and protrudes somewhat--(up to 3 μ) at the posterior end of the body. The undulating membrane is well developed. The costa is delicate, situated somewhat distant from the base of the undulating membrane. The body of the trichomonad is 6.0-15.8 μ long and 4.2-8.2 μ wide.

Trichomonads of the coot (Fulica atra)--fig. 1, 17-19. In fresh preparations most of these trichomonads were oval. Spindle-shaped, pear-shaped, bacilloid and round ones were also seen. In stained smears the cytoplasm of some individuals could be differentiated into ecto- and endoplasm, in most individuals the ventral part stained more heavily. The cytoplasm contains bright colored vacuoles and basophilic granules. The blepharoplasts are small, but well-developed. The 4 anterior flagella of varying length emerge from them in pairs as does the single posterior flagellum, which runs along the undulating membrane. The anterior flagella and free part of the posterior flagellum are about half the length of the body or somewhat more. Trichomonads occur with 2 or 3 anterior flagella. The nucleus is compact, with aggregations of chromatin, more often than not oval, situated somewhat apart from the blepharoplasts. A filament-shaped axostyle is poorly visible in most individuals' cytoplasm and protrudes up to 5 μ at the posterior end of the

body as a sharp spike. The undulating membrane is well developed. The costa is delicate, poorly developed, situated somewhat distant from the base of the undulating membrane. The trichomonad is 7.8-22.7μ long and 4.5-11.3μ wide.

Trichomonads of the grey common pochard (Nyroca ferina)--fig. 2, 20-22. In fresh preparations most of these trichomonads were oval. Pear-shaped and round ones were also seen. In stained smears the cytoplasm was not noticeably differentiated into ecto- and endoplasm. The cytoplasm contains brightly colored vacuoles. The blepharoplasts are large and well developed; from them emerge 4 anterior flagella of varying length and one posterior one. The anterior and the free part of the posterior flagella are about as long as the body or a little longer. Trichomonads with 2 or 3 anterior flagella were seen. The nucleus is ovoid and compact with aggregations of chromatin; it is some distance from the blepharoplasts. The axostyle is tubular, of medium size, poorly stained and emerges from the boundaries of the cytoplasm at the posterior end of the body as a sharp spike (up to 2μ long) only in some individuals. The undulating membrane is poorly developed and passes obliquely from the left to the rear and then to the right. The costa is poorly developed, scarcely noticeable in stained preparations. The trichomonad is 6.2-15.8μ long and 4.2-9.0μ wide.

Trichomonads of the red-crested pochard (Netta rufina)--fig. 2, 23-25. In fresh preparations most of the trichomonads were oval; spindle-shaped, bacilloid and round ones were also found. In stained smears the cytoplasm does not divide into ecto- and endoplasm but does stain intensely; it contains bright-colored vacuoles and basophilic granules. The blepharoplasts are large; from them emerge 4 anterior flagella and one posterior one. The former are approximately as long as the body, while the free part of the posterior flagellum is slightly shorter than the anterior ones. Trichomonads with 2 anterior flagella occur. The nucleus is compact, large, with aggregations of chromatin, more often than not oval, situated at somewhat of a distance from the blepharoplasts. The axostyle is tubular, of average size, stains well and in most individuals emerges (up to 5μ) beyond the cytoplasm at the posterior end of the body as a filament. The undulating membrane and the costa are well-developed. The trichomonad is 6.2-15.2μ long and 4.2-8.4μ wide.

Trichomonads of the grey goose (Anser anser)--fig. 2, 26-28. In fresh preparations these trichomonads are oval. Spindle-shaped and round ones are also seen. In stained smears the ecto- and endoplasm are not noticeably separated. The dorsal part of the cytoplasm in most individuals stains more heavily. The cytoplasm is strongly vacuolated and has a pitted appearance. At the anterior end groups of violet granules (the blepharoplasts) are easily seen. From them emerge 4 free anterior flagella of varying length and one posterior one. Trichomonads are found with 2 anterior flagella. The anterior flagella are somewhat shorter than the body. The nucleus is compact, with aggregations of chromatin, oval, at somewhat of a distance from the blepharoplast. The axostyle is tubular; its anterior end is broadened like a flask; it stains poorly, and only in some individuals protrudes insignificantly at the posterior end of the body. The undulating membrane is very delicate, poorly developed. The costa is well-developed and situated at somewhat of a distance from the undulating membrane. The trichomonad is 6.8-15.8μ long and 4.5-9.0μ wide.

In general the trichomonads described from wild waterfowl are distinguished by their somewhat smaller size, their structure and length of the axostyle, the position of the nucleus and blepharoplasts. Despite this, the trichomonads of wild waterfowl are quite similar to T. anatis of domestic ducks in their number of free anterior flagella, the shape of the body, the comparatively poorly developed undulating membrane, their general location in the intestine of phylogenetically related hosts, etc. Thus we see no basis for separating them into different species. It is possible that further studies will reveal differences at the level of lower taxonomic subdivisions.

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Trichomonad Infections of Wild Waterfowl

Species of Bird	No. Exam.	No. Infected	% Infected
Anas acuta L. (Pintail)	22	11	50.0
Anas platyrhynchos L. (Mallard)	23	10	43.4
Anas strepera L. (Gadwall)	11	5	45.4
Anas clypeata L. (Shoveller)	27	--	----
Anas crecca L. (Teal)	89	40	44.9
Netta rufina Pall. (Red-crested pochard)	24	15	62.5
Nyroca ferina L. (Common pochard)	23	15	65.2
Tadorna tadorna L. (Sheld-duck)	1	--	----
Oxyura leucocephala Scop. (White-headed duck)	6	--	----
Anser anser L. (Gray goose)	12	9	75.0
Nyroca nyroca Guld. (White-eyed duck)	4	--	----
Podiceps caspicus Hall. (Black-necked grebe)	3	--	----
Fulica atra L. (Coot)	79	39	43.0
Gallinula chloropus L. (Moorhen)	11	5	45.4
Larus ridibunduss L. (Black-headed gull)	32	--	----
Chilidonias nigra L. (Black tern)	8	--	----
Ardea alba L. (Great white heron)	2	--	----
Ardea cinerea L. (Common heron)	1	--	----
Phalacrocorax carbo L. (European cormorant)	3	--	----

(ticks, fleas, lice, bedbugs, Diptera) acquire Toxoplasma when allowed to feed on infected animals during the acute phase of the illness (Piekarski, 1949; Laven and Westphal, 1950; Blanc, Bruneau and Chabaud, 1950; Woke, Jacobs, Jones and Melton, 1953; Laarman, 1956, 1957; Nussenzweig and Deane, 1958; Soliman, Rifaat and Morsy, 1963, 1964; Bezukladnikova, Busalayeva, Kusov et al., 1965; Bezukladnikova, 1966, 1968 et al.). And this is wholly possible, since at various stages of their development toxoplasmas are found in the peripheral blood of their host.

Several authors, using other methods of introducing the infectious agent, have obtained positive results. Thiel (1949) and Kunert and Schmidtke (1953) fed the fly Calliphora erythrocephala on a suspension of brain from infected mice or guinea pigs; Weyer (1951) introduced Toxoplasma into the coelom of the louse Pediculus humanus parenterally, and he fed mosquitoes with a mixture of the suspension and blood on a cotton tampon; Woke, Jacobs et al. (1953) infected the louse P. humanus, which sucked blood thru the skin of a chicken; Schmidtke (1955) and Mayer (1962) introduced the peritoneal exudate of a mouse with Toxoplasma into the mouth of the cockroach Periplaneta americana with the aid of a pipette; S. F. Shimansky (1953, 1960, 1961) used the capillary method to infect ixodid ticks, while he used chick embryos to feed infectious material to argasids; Dutkiewicz (1966) introduced Toxoplasma into P. humanus by intrarectal injections using glass capillaries. Our associate V. N. Senotrusova (Bezukladnikova et al., 1965) fed gamasid mites on an exudate plus blood and the larvae of infected fleas. I. K. Teravsky and A. K. Shustrov (1966) infected the tick Ornithodoros papillipes by making it suck blood thru the skin of a tail taken from a white mouse and filled with peritoneal exudate mixed with blood from the liver of the sick mouse, or by introducing peritoneal exudate into the body cavity of hungry ticks, etc.

We checked lice, bedbugs, biting lice (both avian and mammalian) and ants for their ability to contract Toxoplasma while feeding on an infected animal and an infected substrate. The results of the research are presented in Table 1, from which it is apparent that the lice and bedbugs were the most susceptible and the ants were the least susceptible. But even within a group such as the lice several species had infection indices under experimental conditions which varied significantly; in the vole louse H. acanthopus, for example, the infection indices were lower when fed on an infected vole than in the suslik louse N. laeviusculus and the goat louse L. stenopsis when fed on white mice. In experiments on infecting lice and bedbugs, Toxoplasma was isolated in bioprobes on white mice and other positive results were obtained by the CFR.

We used various methods to infect mollusks; freshwater forms were infected with exudate from a toxoplasmosis-infected mouse or with brain with Toxoplasma cysts (the latter were placed in water inhabited by these invertebrates); terrestrial forms were infected by contact, i.e., infected material was placed on green vegetation (grass, leaves) where the mollusks lived, or the infectious agent was administered to them parenterally. The mollusks contracted the Toxoplasma thru the water and by parenteral introduction, but the total percentage of infectiousness proved low.

Thus, on the basis of our own research and the published data mentioned above, we see that the mechanisms and paths of infection for arthropods and mollusks are sufficiently supplied in the case of Toxoplasma. They are varied

to the point where they make it possible for Toxoplasma to exploit these animals as a means of circulation.

Conditions of life and development of Toxoplasma in the vector. As shown in previous studies, all groups of invertebrates proved capable of contracting Toxoplasma. However, the duration of the Toxoplasma life-span and the behavior of the parasites in the bodies of various arthropods remain unclarified.

Many investigators have concluded that Toxoplasma survived in the vector up to 60-70 days (on the average 10-15) in some cases, while most often they last 2-6 days or only a few hours (Table 2).

Weyer (1951) and Woke and Jacobs (1953) have suggested that Toxoplasma lives in the intestinal tract of lice as long as the gut contains unassimilated blood, so that Toxoplasma inside the louse can be supported by further feeding on a warm-blooded host.

The longest survival of Toxoplasma was noted in the ticks Ornithodoros and Dermacentor and the cockroach P. americana. These isolated cases are so far unexplained.

The data on reproduction of Toxoplasma in the body of the invertebrate are contradictory. Splendore (1913) presupposed that sexual reproduction of Toxoplasma occurs in the body of the stable fly. In addition, Chatton and Blanc (1917) felt that the complete life cycle of T. gondii takes place within the body of an infected arthropod. On the other hand, Weyer (1951), Giovannoni and Mello (1952), S. F. Szymanski (1959), Muramoto (1957) and others felt that Toxoplasma does not reproduce in the body of arthropods. We find more precise observations in Weyer (1951), who showed that the Toxoplasma does not invade the cells or organs of P. humanus corporis but is found in the coelomic fluid. Schmidtke (1955), after infecting Calliphora erythrocephala and Periplaneta americana, failed to note any usual or even unusual developmental forms of Toxoplasma in histologic sections of their organs and tissues. Laarman (1957), on the basis of his own research, and in spite of Splendore's findings (1913), also felt that Toxoplasma does not develop in the body of the stable fly. Nor did S. F. Szymanski (1959) find any biological connection between T. gondii and ticks. I. K. Teravsky and A. K. Shustrov (1966) showed that, altho retained for rather a long period in the intestine of Ornithodoros papillipes, Toxoplasma still fails to penetrate into the body cavity of the vector. Moreover, introduced into the body of the tick parenterally, Toxoplasma does not find favorable conditions for development there and dies within two or three days, while organisms isolated prior to that time lose their virulence.

Toxoplasma is also subject to considerable change within the intestinal contents of insects. This was shown in the research done by Nussenzweig and Deane (1958), who observed typical Toxoplasma 16 to 18 days after infection in preparations made from the intestinal contents of infected bedbugs. As early as three or four days, however, they isolated only altered forms of T. gondii; only once (after 12 days) did they find the typical form of Toxoplasma.

In the meantime, Mayer (1962, 1963) in a histologic study of the gut wall of cockroaches 90 minutes after infection with Toxoplasma, found bodies similar to Toxoplasma in the mucosal cells of the esophagus and stomach; after 24 hours

they were also in the muscles of the esophagus. However, he did not see them in any other organs or tissues of the insect and concluded that Toxoplasma is apparently not characterized by migration into the body cavity of the vector. It localizes only in the digestive canal of the arthropod and has no chance to enter the body of the recipient host in the process of the vector's feeding.

The results of these studies bring into question earlier works by Woke, Jacobs, Jones and Melton (1953) in which it was reported that the ticks Rh. sanguineus, D. andersoni, D. variabilis and A. americanus can not only acquire Toxoplasma in bloodsucking but can also transmit it during metamorphosis to succeeding stages of tick development. According to their data, Toxoplasma in D. andersoni survived in the intestine even without undigested blood and passed thru the egg stage, as well as thru the larval and nymphal moults. If these data, obtained more than 15 years ago, had not been strengthened by Dutkiewiez's (1966) recent experiments, it would have been possible to conclude definitely that Toxoplasma is not adapted to habitation in the bodies of arthropods and the result would have been that the basic link in Ye. N. Pavlovsky's scheme--the ability of the agent to survive and develop in the body of the vector--would have fallen out. But Dutkiewicz (1966) found that, introduced artificially into the digestive canal of lice, T. gondii can penetrate into the cells of the intestinal epithelium and hemocytes and multiply in them. He found both typical Toxoplasma and developing intracellular forms in histologic preparations. The reproductive cycle of T. gondii in the cells of the louse did not differ from the cycle observed in the cells of vertebrates. In the cells of the intestinal epithelium and hemocytes of insects Toxoplasma reproduced exclusively by binary division. According to him, the penetration of Toxoplasma into and development within the epithelial cells of the louse intestine resembled closely the development of these protozoa in tissue cultures (Vischer and Suter, 1954; Sourander, Lycke and Lund, 1960; Kaufman and Maloney, 1962). Dutkiewicz's experiments agreed with earlier observations made by Kaufman and Maloney (1962), who showed that in a culture of monkey kidney the "quiet phase" lasts 8-10 hours, during which time the parasites do not reproduce until they have penetrated into the cells. This phase is almost twice as long during the development of Toxoplasma in the intestine of the louse.

Dutkiewicz observed how longitudinal division of T. gondii in the epithelium of the louse intestine is accompanied by an increase in size prior to division by the presence of the characteristic forms and the joining together of the latter at their ends after division--which had been described previously by several authors (Hirschlerowa and Kozar, 1952; Pulvertaft, Valentine and Lane, 1954; Thalhammer, 1957). Dutkiewicz wrote not only about the behavior of Toxoplasma in the intestinal wall of insects, but in further studies he proved that Toxoplasma is present in the hemolymph, where it is found intracellularly; he ascribed this to active penetration of the protozoa from the intestinal lumen thru the epithelium and into the body cavity. Here too he introduced considerable variations in the form and dimensions of the Toxoplasma, which depend on the surrounding environment. He thought that clusters of Toxoplasma can appear under natural conditions and intra-cellular reproduction in tissues of infected arthropods, creating favorable conditions for the infection of healthy hosts by the eating of infected insects or by the flesh of infected insects falling into a wound after a bite. However, there is not a

word here about the division of Toxoplasma during the course of metamorphosis in arthropods as Woke et al. (1953) bravely reported. Nor did Dutkiewicz express any thought as to whether Toxoplasma may penetrate into the body of the recipient with the saliva of the arthropod when it sucks blood, which in general differs only slightly from these preceding studies.

Conclusive solution of this problem is represented by the results of studies on spontaneous harboring of Toxoplasma by arthropods. Unfortunately, there is still very little information on this subject. Many studies of the natural harboring of Toxoplasma by gamasid, ixodid and argasid ticks, blood-suckers, bedbugs, lice as well as fleas have given negative results (Pixell, 1913; Chatton and Blanc, 1917; Giroud, Grjebine, 1951; Gibson and Eyles, 1957; Pope, Bick and Cook, 1957; Cardenas Vasquez, 1958; Rifaat, Saliman and Morsy, 1963). Only Giroud and Grjebine (1951) reported isolating a strain of Toxoplasma from Rhipicephalus sanguineus taken from a dog. The same worker in conjunction with other authors (Giroud, Le Gac and Gailard, 1952) reported isolation of Toxoplasma from the bodies of red mites Trombicula legaci taken from rats. He, too (1961), succeeded in isolating T. gondii twice from flies caught in the laboratory.

These cases, always by the same people, require confirmation. And finally, Gidel and Provast (1965) succeeded in isolating Toxoplasma from naturally infected ixodid ticks of the genus Amblyomma.

3. Virulence of Toxoplasma in the invertebrate body. In the body of an invertebrate the temperature depends on the temperature of the surrounding environment, so that the virulence of Toxoplasma changes. P. A. Petrishcheva (1967), for example, felt that the variability of a disease agent may display itself to a greater degree in the body of bloodsucking arthropods than in the vertebrate body. On the other hand, when weakened strains of pathogenic microorganisms were used in experiments she observed that bloodsucking arthropods were almost immune to them or had a very slight susceptibility. She felt that it is the weakened strains of microorganisms which circulate considerably more often in nature. Galuzo et al. (1966, 1968) came to the same conclusion in considering the question of Toxoplasma virulence.

Various authors working under laboratory conditions with the vectors of Toxoplasma have tended most often to use the virulent RH strain.

Trying to infect P. humanus with Toxoplasma in the body cavity, Weyer (1951) showed that Toxoplasma retained its virulence in the coelomic fluid for 13 days; according to Woke et al. (1953), Toxoplasma is able to remain virulent in the louse intestine for five to seven days if the intestine contains undigested blood; they can last no more than six days in the intestines of the bugs R. prolixus, T. phyllosoma and T. rubrofasciata. However, the same authors (1953) noted a decrease in the virulence of strain RH as compared with its initial level when it passes thru Rh. sanguineus ticks; this the authors concluded from the survival rate of white mice inoculated with a suspension of such ticks.

Kunert and Schmidtke (1953) indicated that Toxoplasma when passed thru the intestines of flies loses its virulence.

Jacobs and Melton (1954) conducted observations on the change in virulence of strain 113, isolated from Rh. sanguineus. This strain was of low virulence, but after passing in thru a chicken embryo and the body of an intracerebrally inocculated white mouse, its virulence was raised.

Mraz (1957, 1959) asserted that the virulence of Toxoplasma during passage thru ticks of various species drops irrevocably, as shown by a lengthening of the asymptomatic period and of the survival time of mice after infection. S. F. Szymanski (1958, 1959, 1960, 1961) noted the same phenomenon in Toxoplasma isolated from sick infants and animals and then passed thru ticks. I. K. Teravsky and A. K. Shustrov (1966) explained that when Toxoplasma is introduced parenterally it loses its virulence and dies in two or three days.

Deane and Nussenzweig (1959) advanced the hypothesis that there are other (besides proliferative) forms of Toxoplasma which do not cause acute illness in white mice and do not cause a positive dye test. These forms may survive in the digestive tracts of bloodsucking arthropods.

In our own work we used both markedly virulent and avirulent strains of Toxoplasma (see Table 1). Here too it should be pointed out that the percentage of infection of invertebrates in the experiments with virulent strains of Toxoplasma was significantly higher than in experiments with the avirulent strains. We can get some idea as to the virulence of the Toxoplasma from the survival rate of the white mice infected thru arthropods. White mice were used in every experiment with bedbugs, Mallophaga, lice, and mollusks which had been infected with virulent or avirulent Toxoplasma. The mice survived, or, more properly, they relapsed into a light, chronic form of the disease. In rare cases they died, but we are inclined to ascribe those cases to other causes. The virulence of the markedly virulent strains decreased irrevocably. As confirmation of this we can cite the results of our studies showing that basically all the white mice gave positive readings on the CFR. In almost 400 experiments with invertebrates there were 74 cases (18.5 per cent) in which white mice yielded positive CFR's at low titer (1:5) and only 13 cases (3.2 per cent) in which Toxoplasma was isolated from the mice.

On the basis of his own research Weyer (1951) came to the conclusion that T. gondii has no effects on the tissues of arthropods. The deaths he observed among lice infected with Toxoplasma he ascribed to intoxication of the lice as a result of the death and decomposition of the Toxoplasma. Dutkiewicz (1966), on the other hand, believed that the death of the lice after five or more days was due to destruction of the tissues of these insects as a result of intense Toxoplasma multiplication. We observed a somewhat higher than usual death rate among lice (H. acanthopus, L. stenopsis, N. laeviusculus) when they were fed on an animal infected with Toxoplasma. Similar phenomena have been noted in mollusks.

4. Mechanism and conditions of transmission of the agent to the recipient host. The question as to whether infected arthropods can transmit Toxoplasma while feeding on a healthy animal is a basic one in all works concerning the vectors of this organism. Experiments with argasid and ixodid ticks, bedbugs, fleas, flies, mosquitoes and bloodsuckers have in the overwhelming majority of instances yielded negative results (Carmio, 1933: Laven and Westphal, 1950; Jacobs, Woke and Jones, 1950; Blanc and Bruneau, 1950; Weyer, 1951; Havlik, 1951; Woke et al., 1953; Giovannoni et al., 1952-1954; Thiel and Laarman,

1953; Nakajvo, 1957; Mraz, 1957, 1959; Deane, 1958; Szymanski, 1959; Varela and Zavala, 1961; Pestre et al., 1962).

Woke, Jacobs, Jones and Melton (1953) obtained positive results in transmitting Toxoplasma thru the bite of P. humanus corporis lice, and also in experiments with the ticks D. variabilis, D. andersoni and A. americanum. Laarman (1956) registered one case in which a guinea pig was infected thru the bite of a stable fly S. calcitrans. Deane (1958) infected a healthy white mouse by placing upon it a female A. cajennense which had been taken 17 days before, half-engorged, from a white mouse that had died of acute toxoplasmosis. However, he doubted whether the infection really got in thru the bite or whether it arose after feces or some other excretion of the tick was carried into the wound. Nussenzweig and Deane (1958) infected white mice with Toxoplasma thru bites of nymphal bugs Rh. prolixus.

A positive CFR in experiments with fleas was botained by N. N. Buslayeva (Bezukladnikova, Buslayeva et al., 1965), who succeeded in infecting white mice by feeding them on infected fleas.

In our experiments, the feeding of infected L. stenopsis and H. acanthopus lice on healthy white mice led to positive results on the CFR in a significant percentage of experiments. One white mouse reacted positively after 70 infected C. lectularius bedbugs fed on it.

Alimentary transmission thru vectors has been described by Laven and Westphal (1950). They established that the infection can be transmitted when animals eat Nosopsylla fasciatus fleas. Woke, Jacobs, Jones and Melton (1953) obtained positive results in peroral infection of white mice with a suspension of the bodies of P. humanus lice; Kenert and Schmidtke (1953), in experiments with Stomoxys (they fed white mice a mixture of milk and fly intestine), also were successful. Nussenzweig and Deane (1958) succeeded in transmitting Toxoplasma to two white mice which had eaten the nymph and imago of Rh. prolixus 72 hours after the latter had had an infectious meal. However, Kozar (1959), in experiments designed to clarify the transmission of Toxoplasma thru infected insects (Silphidae) to white mice by the alimentary route, obtained negative results, as did S. F. Szimanski (1959) in experiments with A. persicus ticks.

Our associates have obtained positive results by feeding white mice the bodies of infected O. lahorensis nymphs, the larvae of C. canis fleas and the imagos of L. segnis fleas (Bezukladnikova, Busalayeva and Kusov, 1965).

In our studies with three species of lice (L. stenopsis, N. laeviusculus, H. acanthopus) and bedbugs (C. luctularius), which we administered to white mice per os, several experiments yielded a positive CFR, but no Toxoplasma could be isolated from the subject mice.

Considering that, when a parasite bites, the skin is wounded, and that if the insect is crushed its contents inevitably get into the wounded area, some authors (Havlik, 1951; Dutkiewicz, 1966 and others) have expressed the opinion

that infection can be transmitted thru contamination of small skin injuries. We arranged a series of special experiments to test whether transmission of Toxoplasma can occur when the body contents of infected insects touch the scarified skin of an animal: a suspension of the bodies of engorged lice L. stenopsis, H. acanthopus and N. laeviusculus was poured upon or rubbed into damaged skin. In all, the experiment dealt with more than 1000 lice on 33 white mice. The result was that seven mice were positive to the CFR at a dilution of 1:5 (Table 3); in no case was Toxoplasma isolated from any animals.

As to the possibility of Toxoplasma being transmitted by arthropods thru contamination, an important factor is represented by the routes Toxoplasma follows in its excretion from the body of the vector. Thiel (1949) showed that the flies C. erythrocephala, which acquire Toxoplasma by eating food containing the parasites, excrete it from their bodies by eructation. T. gondii survives up to two hours in the saliva of this insect. Havlik (1951) found Toxoplasma in the coxial fluid and feces of the tick O. moubata 23 days after the infectious feeding. Positive results were obtained in 4 out of 9 fecal tests on D. andersoni ticks (Woke et al., 1953). Kunert and Schmidtke (1953) found Toxoplasma in fresh fly saliva and in a suspension of their heads and crops 24 hours after infectious feedings and in the feces 48 hours afterwards.

In Muramoto's (1957) studies white mice infected thru cockroach feces containing Toxoplasma for 10 hours reacted positively. According to Varela and Zavala (1961), Toxoplasma was found in the feces of fleas and flies, while feeding mice on food contaminated with feces on infected flies, or on the feces themselves, yielded a positive result.

N. N. Busalayeva (Bezukladnikova, Busalayeva et al., 1965) obtained positive results on the CFR with a bioprobe from the guano of L. segnis fleas, while we did the same thing in a bioprobe of the feces of B. lantzi mollusks. These data speak in favor of the fact that Toxoplasma can be transmitted by contamination of wounds on the recipient with guano, coxal fluid and other excreta and discharges of infected insects and arthropods.

5. Mechanism by which Toxoplasma is transmitted by Toxocara cati nematodes. The recent studies by Hutchison (1965, 1967) and Dubey et al. (1968) on transmission of Toxoplasma by nematodes deserve special attention. Hutchison (1965, 1967) has shown that Toxoplasma is associated with the intestinal nematode T. cati and emerges into the external environment with its eggs. In the process of the eggs' development it is preserved, enters the larvae and ends up with the larvae in the stomachs of warm-blooded animals. Here it is noted that only development of the egg can cause mice to become sick with toxoplasmosis when they are fed Toxocara eggs. The main thing is that, once inside the eggs, Toxoplasma can survive outside the host for as long as 17 months, depending on environmental conditions, tho not all nematodes furnish the conditions necessary for survival. Jacobs and Melton (1966) found cats naturally infected with Toxoplasma and T. cati. Dubey (1968), having confirmed in general the studies made by Hutchison, showed by complex experiments the circulatory mechanism of Toxoplasma in Toxocara, its entrance into the body of the helminth, its behavior inside and its ultimate emergence.

Toxocara eggs, like the body of the nematode, are covered with a solid, hard wall--a cuticle--whose penetration is hardly possible for Toxoplasma. Thus the authors concluded that Toxoplasma enters the egg prior to formation of the wall in the ovary. Toxoplasma gets into the body of the helminth along with its food. Hutchison thought that the intestinal nematodes digest the food found in the digestive tracts of their hosts. When this food contains Toxoplasma cysts, they along with the food get into the body cavity of the nematode and there (after digestion) become liberated. He supported this idea with data from Jacobs, Remington and Melton (1960). The helminths in the host's intestine have an opportunity to obtain live Toxoplasma (trophozoites) per os as soon as they are freed from their cysts in the infected food. Since the nematodes found in the stomach migrate into the small intestine or farther, they have an opportunity to engulf along with their food the trophozoites which have penetrated into the intestine, and by the same token the possibility increases that Toxoplasma will enter the body cavity of the helminth. Dubey, continuing Hutchison's idea, thought that if Toxoplasma has entered the nematode's intestine its migration thru the helminth's intestinal wall and body cavity to the ovary is not a long process and involves no special difficulty. In a system of complex experiments he showed that as the larva develops in the egg the Toxoplasma enters its body and lives on its metabolites.

It is hard to say how many toxoplasmas one Toxocara egg may contain; however, Dubey infected mice with Toxoplasma after giving them two infected Toxocara larvae. Hence the question has arisen: Does Toxoplasma reproduce inside the Toxocara? In order for transmission of Toxoplasma by Toxocara to occur, the helminth eggs must complete the embryonic period; altho undeveloped eggs contain Toxoplasma, they cannot transmit it to another host. If Toxoplasma develops inside the Toxocara, then where and how does this process take place? Confronting the two factors necessary for development of Toxoplasma--the presence of aerobic conditions and an ambient temperature of around 37°--Dubey admitted that its development is possible only during the migration of the larva.

No one has as yet obtained anatomic evidence of the presence of Toxoplasma in the nematode's body or of its eggs in the larvae. The possibility is not eliminated that the Toxoplasma found developing inside a helminth may have another form in addition to the cyst and trophozoite.

No less important is the question as to how Toxoplasma leaves the body of the Toxocara larva and how it penetrates into the body of the vertebrate host. In larvae, as is known, there are no alimentary or excretory canals thru which Toxoplasma could leave the body. It is suggested that Toxoplasma kills the larva. The body of the larva collapses and the Toxoplasma penetrate into the body of the vertebrate host.

The whole complex path covered by Toxoplasma in the helminth's body has no effect on the former's viability or virulence. The course of a toxoplasmosis infection in mice infected by means of Toxocara proved the same as in mice infected by other methods.

The course of experiments with Toxocara recounted here and their results agree with Ye. N. Pavlovsky's conception of the parasitocenose. T. gondii in the process of evolution "found" Toxocara cati among the components of its

parasitocenose and then proceeded to exploit it as a means of changing hosts and as a habitat of the second order for its development.

Actually, Toxocara is a cosmopolite, which agrees with the worldwide distribution of Toxoplasma. Because of their life style, nematodes can transmit Toxoplasma not only among animals but from animals to man. Woodruff (1965) showed that the larvae of several Toxocara species can migrate within the human body. Toxocara is found in the retina of the eye and in other human organs and tissues. The cosmopolitan distribution of Toxocara species, their presence in carriers of Toxoplasma (the ability of Toxocara to infect a wide circle of homoiothermic animals) is an ecologic prerequisite for these helminths to become vectors of Toxoplasma.

Studies in this direction have been carried out with other species of helminths too (Vermeil and Marquet, 1967; Tikhovskaya et al., 1968: Mgaloblishvili, 1968; Kheysin et al., 1969).

In recent years a new form of Toxoplasma has been discovered in cat feces; it is somewhat different in both biologic and anatomic characteristics from the usual Toxoplasma cysts (Work and Hutchison, 1969). These authors feel that the fecal cysts, being resistant in the external environment, may serve as a source of infection for susceptible animals.

The role of parasitic arthropods as vectors of Toxoplasma appears somewhat different to us. The whole complex of studies does not answer the demands of Pavlovsky's triad: it has been proven that Toxoplasma can enter the body of an invertebrate and at the same time its "helplessness" in the vector's body has been shown--it cannot pass beyond the intestine, and if, according to some data, it does pass on, it does not reach the exit route--the salivary-oral apparatus. And positive experimental transmission by inoculation is extremely rare and not very convincing. Along with this it has been shown possible for an animal to become infected by the oral route or by contamination. In other words, if a parasite, having gotten into the body of an arthropod, has not died, it can be transmitted mechanically to another animal, keeping in mind that for each vector there is a limited range which does not necessarily enter into the range of the agent. Despite the series of positive experiments reported above, it is not possible to recognize parasitic arthropods as a natural link in the circulation of Toxoplasma in nature. It is possible only to admit them as a purely epidemiologic or epizootiologic factor aiding in the circulation of the agent inside a limited nidus in nature or daily life.

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Table 1

Results of infection of invertebrates by *Toxoplasma* (authors' data)

Species	Number studied	Number of experiments	Strain		Number of positive experiments	Positive experiments in which *Toxoplasma* was isolated
			Virulent	Avirulent		
Bedbugs:						
Cimex lectularius	141	8	CFL	-	2	1
"	77	4	CLN	-	3	1
Mammalian biting lice:						
Trichodectes caprae	730	9	ODG-2	-	3	-
"	300	3	-	VFG-4	1	-
Trichodectes bovis	1200	6	-	VFG	1	-
Gyropus ovalis	28	1	CFL	-	Neg.	
Gliricola porcelli	135	1	-	LTI	"	
"	180	2	CFL	-	1	-
"	150	1	RH	-	Neg.	
"	100	1	GGP	-	"	
"	45	1	VVN	-	1	
Avian biting lice:						
Menopon gallinae	320	5	ADP	-	1	-
Eomenacanthus stramineus	80	1	"	-	Neg.	
Sucking Lice:						
Enderleinellus suturalis	412	3	CFL	-	1	1
Linognathus vituli	100	1	ODG	-	Neg.	-
"	260	3	VFG	-	"	-
Polyplax spinulosa	44	2	CFG	-	1	1
"	231	3	LEL	-	3	-
Polyplax serrata	370	2	VFG	-	1	-
"	100	3	ALG	-	Neg.	-
Neohaematopinus laeviusculus	2034	18	CFG	-	Neg.	-
"	130	2	LEL	-	1	-
Hoplopleura acanthopus	34	1	VVL	-	1	-
"	78	2	SDK	-	1	-
"	60	2	CFL	-	1	-
"	6019	50	LEL	-	12	1
Linognathus setosus	12	2	CDN	-	17	1
Linognathus stenopsis	392	9	ODG-2	-	3	-
"	768	26	CFL	-	17	-
Ants:*						
Lasius niger	100	1		OCG	1	-
"	700	7	RH	-	1	-
Formica pratensis	100	1	SHP	-	Neg.	-
"	500	5	RH	-	Neg.	-
"	1700	17		ALG OCG LTI	Neg.	-
Mollusks:						
Bradybaena lantzi	590	34	RH	-	1	-
"	72	18	HMK	-	2	-
"	84	15	HSK	-	2	-
"	163	17	LEL	-	3	2
"	80	8	CFL	-	Neg.	
"	75	4	ADP	-	1	1
"	90	6	ODG	-	Neg.	1
"	23	4	FDN	-		-
"	410	9	STG	-		-
"	95	5	SDK	-	1	-
"	13	2	CDG	-	1	-
"	248	3	-	OCG	1	-
"	100	2	-	AIG-4	1	-
"	189	37	-	VFG-4	2	-
"	484	21	-	LTI	2	-
Radix pereder	1	1	-	VFG	Neg.	-
Lymnaea iliensis	15	5	-	"	"	-
"	6	2	-	LTI	1	-
Coretus corneus	9	3	-	VFG-4	1	-
"	3	1	-	LTI	Neg.	-
Physa acuta	3	1	-	VFG-4		-
"	3	1	-	LTI		-

*Determination performed by P. I. Marikovsky

Table 2

Duration of *Toxoplasma* survival in invertebrates

Species	Hours	Days	Author, year of publication
Ticks			
A. persicus		20	Szymanski, 1959
O. moubata		23	Havlik, 1951
O. lahorensis		20,65	Szymanski, 1959; Bezukladnikova, Busalaveva and Kusov, 1965.
O. papillipes		21	Szymanski, 1959
O. coniceps		15	"
Rh. sanguineus	Several	-	Blanc et al., 1950
D. pictus		60*	Woke et al., 1953
"		5	Szymanski, 1959
D. marginatus		3	
D. variabilis		5	Woke et al., 1953
D. andersoni		4.70*	
H. asiaticum		5	Szymanski, 1959
Dermacentor, Haemaphysalis		30	Frenkel, 1962
Cockroaches			
P. americana		65	Mayer, 1962
Lice			
P. humanus		5-13	Weyer, 1951; Woke et al., 1953; Dutkiewicz, 1966
N. laeviusculus	24		Our data
Bedbug			
C. lectularius	5	4, 3	Piekarski, 1949, Varela and
Assasin bug		3	Zavala, 1961
			Our data
Rhodnius sp.	5	20	Piekarski, 1949; Deane, 1958
Fleas			
X. cheopis	Several		Blanc et al., 1950
N. fasciatus		13	Laven and Westphal, 1950
Ct. segnis		21	Piekarski, 1949
Ct. canis		7	Bezukladnikova, Busalayeva et al., 1965
Diptera			
S. calcitrans	Several		
C. erythrocephala		4	Thiel, 1949
An. albimanus	Immediately After		Varela et al., 1961
C. quinquefasciatus		4	Giovannoni et al., 1952-1954
Ked			
M. ovinus		9	Pestre et al., 1962
Mollusk			
B. lantzi		15	Our data

*In cases of trans-phase transmission

Table 3

Results of investigations into the mechanism of Toxoplasma transmission

Species	Strain	Number of insects examined	Number of experiments	Method of running the experiment	Number of positive experiments
Bedbugs					
C. lectularius	CFL	59	1	per os	1
"	"	89	2	Brought in contact with scarified skin	None
"	CDN	46	3	Thru bite	1
Lice					
N. laeviusculus	CFG	210	4	per os	2
"	"	200	4	Brought in contact with scarified skin	3
"	"	530	2	Thru bite	None
H. acanthopus	LEL	1234	18	per os	3
"	"	1372	13	Brought in contact with scarified skin	1
"	SDK	78	1	"	None
"	CFL	60	1	"	1
"	LEL	1020	9	Thru bite	4
L. stenopsis	CFL	141	5	per os	4
"	"	115	3	Brought in contact with scarified skin	1
"	ODG	170	3	"	1
"	CFL	101	5	Thru a bite	4