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THE RELATIONSHIPS BETWEEN PROTOZOA AND VIRUSES

3. PROTOZOA AS INACTIVATORS OF VIRUSES

Besides studying endogenic viruses, virus-like particles (VLPs) and viral cytopathogenic agent in protozoa, dealt with by the authors in the series of papers (Teras, Kesa, 1988a, b), a number of protozoologists and virologists have made attempts to elucidate the relationship between protozoa and mammalian viruses. At that the following has been established: a) protozoa can inactivate (*resp.* destroy) mammalian viruses, and b) mammalian viruses can replicate and persist in the organism of protozoa.

In the present paper the authors will give a survey of the former phenomenon based both on data found in literature and the experimental results of their own.

P. Groupé and L. Pugh (1952) were the first to ascertain that protozoa can inactivate mammalian viruses. In their experiment they used an axenic culture of *Tetrahymena geleii* (synonym for *T. pyriformis*), to which they added allantois liquid from chick embryos, infected with type A (strain PR/8) or type B (strain Lee) of influenza virus. Simultaneously with the experiments on living cultures, tests were carried out on killed cultures of the ciliates and influenza virus was studied also in the allantois liquid which did not contain protozoa.

Samples were taken both from the system and control cultures, hemagglutination reaction was performed and the infectious titer of the virus on chick embryos established. After 48 hrs the authors found that the infectious titer of the virus in system cultures was much lower than in the control medium, which contained only the virus. As the results of the experiments with killed and living ciliates appeared to be much the same, the authors supposed at first that lessening of the virus titer was due do some factors of the ciliates which can inactivate the influenza virus. But additional experiments revealed that all the particles of the virus were inactivated or decomposed only in the cultures containing intensively multiplying ciliates. The phenomenon of inactivation established is still based on the ability of *T. pyriformis* to phagocyte influenza virus (Groupé et al., 1955).

Using *T. pyriformis* beside the cultures of *Glaucoma scintillans*, *Euglena gracilis* and *Astasia klebsi*, the authors ascertained that the inactivation of influenza virus in the cultures of *T. pyriformis* takes place very quickly, whereas the titer of hemagglutinins of the virus dropped from 1:512 to 1:32 already during the first 10 hrs. In the control experiments where influenza virus was incubated in the medium, used for cultivating *T. pyriformis*, its titer remained at the initial level (1:512) during the whole period of observation (for 48 hrs). According to V. Groupé et al. (1955) *T. pyriformis* inactivated influenza virus as quickly as the Californian strain of NDN virus of the Newcastle disease. But in the analogical experiments with the free-living protozoa *E. gracilis*, *A. klebsi*, *G. scintillans* and influenza viruses the authors did not observe the ability to inactivate these viruses. In their opinion it could be explained by the absence of oral foramen in the two first species, and the inability of

G. scintillans to digest phagocytosed viruses due to which viruses escape untouched.

On the basis of the results obtained V. Groupé et al. expressed the opinion that free-living protozoa may play an important role in purifying sewage water from viruses, and that the protozoa can be used instead of bacteria as food for obtaining axenic cultures of some species of ciliates.

A year later after the publication of the work of V. Groupé et al., M. Knorr et al. (1956) attempted to elucidate the possible replication of influenza virus and coliphages in amoebae of *Naegleria* family, isolated from drinking water. In their experimentally created systems they observed inactivation of influenza virus A and cowpox virus. A notable lessening of infectiousness was detected after 12 hrs had passed from the beginning of the experiment. But as amoebae in analogical experiments did not inactivate the coliphage, the authors were the first to suggest that while investigating relationships between protozoa and viruses, one should always consider the possibility of the existence of indifferent systems of protozoon + virus, and that especially when interpreting the results, obtained at studying replication of viruses in protozoa. The experiments carried out by L. Bauer (1961) show, however, that indifference in associations between protozoa and viruses is relative, as among amoebae there are also such species which are able to inactivate the coliphage as well. While examining the reasons of disappearance of the phage from cultures of amoebae *Vahlkampfia limax*, Bauer established that the phenomenon is due to the ability of the latter to intensively inactivate the given virus. With the help of electron microscope the author successfully demonstrated that inactivation of the coliphage takes place just in the organism of amoebae, and not in the environment under the influence of some extracellular enzyme or amoebial metabolic products. According to Bauer *V. limax* intensively inactivates also influenza virus A and cowpox virus, added to cultures of protozoa. In these cases, too, the author could observe the process of lysis of these viruses in the organism of *V. limax*.

In order to investigate the relationships between protozoa and viruses F. Blawat and Z. Kowalska (1963) used cultures of amoebae. Their aim was to elucidate the fate of enteroviruses (first of all that of poliovirus) in sewage water, soil and drinking water. That is why the authors chose for their experiments cultures of *Acanthamoeba castellanii*, living in the soil, *Entamoeba moshkovskii*, frequently occurring in drinking and sewage water. Having added a certain amount of poliovirus type 1 (stock Brunhilde) or type 3 (stock Saukett) to the cultures of these two protozoan species, they observed the created systems for 42 days. To determine infectiousness of poliovirus cell cultures Detroit 6 or HeLa were used, depending on the strain of the virus.

Lessening of poliovirus' titer was observed in cultures of both species of amoebae. In cultures of *A. castellanii* the poliovirus totally disappeared in 18—21 days, whereas the authors regarded this as natural denaturation of viral protein. At the same time from cultures of *E. moshkovskii* the poliovirus disappeared very quickly. After 24 hrs the infectiousness of the poliovirus was by 30 per cent lower than in the control medium, containing only viruses. According to the authors inactivation of poliovirus was due to phagocytosis of viral particles by amoebae, which, most probably, used them as food. To that testify the authors' observations that in meagre media, such as Pavlov's, inactivation of the virus occurs sooner than in Lb medium which contains pepton and yeast extract.

This observation is, doubtless, very important in carrying out experimental investigations of relationships between protozoa and viruses, since using different media for cultivation of unicellular organisms may lead to different results.

The phenomenon of inactivation of viruses by free-living protozoa and, especially, inactivation of influenza virus by *Tetrahymena pyriformis*, was confirmed also by other authors (Teras et al., 1974; Терас и др., 1981; Perez-Prieto, Garcia-Gancedo, 1975). But as far as we know, only M. Chýle et al. (1971) have ascertained this ability also in parasitic protozoa. According to them, after adding suspension of pseudorabies virus to axenic cultures of *Trichomonas vaginalis*, absolute inactivation of the virus followed in 48 hrs. At the same time the authors observed acceleration in propagation of the protozoa as well as activity of their isoenzymes lactatdehydrogenase, malatdehydrogenase and glutamatdehydrogenase (Chýle et al., 1985).

The same form of relationship between protozoa and viruses was investigated also by us whereas we also succeeded in getting some additional information about the relationship between influenza virus and *Tetrahymena pyriformis*.

Beside four well-known laboratory strains of the human A-type influenza virus (A/Vic/36/72 (H₃N₂); A/Aichi/1/68 (H₃N₂); A/WSN/33 (H₁N₁) and A/PR/8/34 (H₁N₁)) we also used in our work 3 local strains of A-type, recently isolated from patients, one strain of bird-type (A/FPV/Rostock/34/ (H₇N₇)), one strain of swine-type (A/SW/Wisc/68 (H₁N₁)) and one strain of seal-type (A/Seal/Mass.1/80 (H₇N₇)). It occurred that *T. pyriformis* was able to eliminate all the investigated types of influenza virus. Thus, in the all experimentally *in vitro* created systemic cultures of *T. pyriformis* + influenza virus the initial hemagglutinative titer of the virus dropped in no more than 7 days below the definable value (1:2). In the control medium both the infectious and hemagglutinative titer of the virus remained unchanged during the whole period of observation (Table 1).

Table 1

Data on the dynamics of the hemagglutinative titer of different types of influenza virus in the supernatant of the cultures of *T. pyriformis*, incubated in allantois liquid

N of experiment	Influenza virus		Hemagglutinative titer of the virus in the supernatant							Hemagglutinative titer of the virus in allantois liquid containing only virus	
	type	host	at the beginning of the experiment	after							
				24	48	72	96	120	144		168
				hours' incubation with <i>T. pyriformis</i>							
1.	A/Vic/75 (H ₃ N ₂)	man	512	128	32	<2					512
2.	A/Tallinn/20/75/	man	128	128	64	64	32	16	8	<2	128
3.	A/Tallinn/25/75/	man	128	128	64	72	8	<2			128
4.	A/Tallinn/27/75/	man	256	256	128	128	64	16	8	<2	256
5.	A/Aichi/1/68 (H ₃ N ₂)	man	512	<2							512
6.	A/WSN/33 (H ₁ N ₁)	man	512	16	<2						512
7.	A/PR/8/34 (H ₁ N ₁)	man	512	16	<2						512
8.	A/FPV/Rostock 34 (H ₇ N ₇)	bird	512	32	<2						512
9.	A/SW/Wsc/68/ (H ₁ N ₁)	swine	512	<2							512
10.	A/Seal/Mass./1/80 (H ₇ N ₇)	seal	512	<2							512

Note: The hemagglutinative titers have been given in reciprocals.

Being convinced that in the system *T. pyriformis* + influenza virus the latter was quickly eliminated, we tried to find out whether succeeding populations of the ciliates, having had contact with influenza virus, were also able to destroy the virus. For that purpose we used the ciliates, centrifuged from the systemic culture of *T. pyriformis* + influenza virus whereas the hemagglutinative titer of the virus had already dropped to 1:2, and washed three times in 0.85 per cent solution of NaCl. The ciliates were then sown in allantois liquid of chick embryo which contained influenza virus with hemagglutinative titer of 1:512.

We did altogether nine such repeated passages of *T. pyriformis* into allantois liquid containing influenza virus whereas at each passage we ascertained the hemagglutinative titer of the virus in the supernatant during 7 days.

The results (shown in Table 2) were rather surprising, for it appeared that although the ability of *T. pyriformis* to eliminate influenza virus remains even after repeated contacts with this virus, some subcultures of ciliates may fail to eliminate influenza virus. Thus we did not observe on the part of *T. pyriformis* any restraining effect on the titer of influenza virus in two successive systemic cultures (the 4th and 5th passages), which in next passages, however, became even more intensive than before the unexpected "refusal" of *T. pyriformis* to eliminate influenza virus. We could not find out the reason for that phenomenon, therefore we may only suppose that it was due to a temporary stoppage in the virus-splitting ferment system of *T. pyriformis* that should be taken into account in further investigations of the relationship of protozoa with viruses.

Table 2

Data on the hemagglutinative titer of the influenza A/Vic/36/72/(H₃N₂) virus in the supernatant of the cultures of *Tetrahymena pyriformis*, incubated in allantois liquid and having repeatedly been in contact with influenza virus

The investigated culture of <i>T. pyriformis</i>	Hemagglutinative titer of the virus after						
	24	48	72	96	120	144	168
Initial culture with influenza virus	128	128	64	32	<2		
The 2nd passage with influenza virus	256	128	8	<2			
The 3rd " " " "	256	128	128	32	32	8	<2
The 4th " " " "	512	512	512	512	512	512	512
The 5th " " " "	256	256	256	256	256	256	256
The 6th " " " "	512	512	512	512	64	<2	
The 7th " " " "	256	32	<2				
The 8th " " " "	64	<2					
The 9th " " " "	32	8	<2				
The 10th " " " "	32	<2					
Control medium containing only influenza virus	512	512	512	512	512	512	512

Note: The hemagglutinative titers have been given in reciprocals.

Interesting data on inactivation of viruses by protozoa have provided also some Spanish authors (Perez-Prieto, Garcia-Gancedo, 1978, 1979, 1981; Perez et al., 1985; Jareño et al., 1980), who used *T. pyriformis* as a model in their first experiments, but later also another species of free-living ciliates, namely *Onychodromus acuminatus*. For experiments they

used cultures of ciliates, having been in contact with viruses for different periods of time. At the same time with observing virus in the supernatant of the systemic cultures by virological methods, the virus was studied in the ciliates also by electron microscopy as well as in cell cultures while using repeated freezing and thawing for crushing ciliates.

At studying first by such methods the relationship of *T. pyriformis* with vaccinia virus, it occurred, that the virus, the titer of which remained unchanged in a ciliate-free control medium during 11 days, was detectable only within 24 hrs in the supernatant of the cultures, incubated at either 25° or 37°C.

But in the crushed ciliates the virus could be found during all the period of observation, i. e. 11 days, whereas 4 first days after creating the system protozoon+virus the titer of the virus showed rapid and constant fall, thereafter rise. At the end of the observation period the titer of the virus reached again almost the same value as on the 1st and 2nd day of the experiment. By electron microscope the virus particles with well preserved structure could be seen only during a short period (25 minutes) in nutrient vacuoles near the surface of *T. pyriformis* which had been in contact with vaccinia virus, whereas numerous mitochondria were around.

On the basis of these results the authors (Perez-Prieto, Garcia-Gancedo, 1981) came to the conclusion that *T. pyriformis* has an ability to pass the vaccinia virus that happens to be in its nutrient medium quickly and, probably even selectively, through its oral cavity to nutrient vacuoles where the virus is gradually destroyed. In case the quantity of the virus consumed is big, it may, according to the authors, persist in the organism of the ciliates for some time and is likely even to replicate there, whereas it is not impossible that the genome of the virus preserves in protozoa for quite a long period.

The Spanish authors tried to prove the hypothesis of persisting of the viruses in the organism of protozoa, which had inactivated them, also in their following papers (Perez et al., 1985). They have reported the results on analogical experiments on the relationship of *T. pyriformis* with Sinbis, vesicular stomatitis, herpes 1 and herpes 2 viruses, as well as with some types of enteroviruses. The latter, regardless of their disappearance from supernatants of the systemic culture of protozoon + virus in 48 hrs at the latest, were in lysates, made from the protozoa, BGM cells or fibroblasts of chick embryo, detectable even on the 30th day of the experiment.

Unfortunately next tests, carried out on another free-living ciliate — *Onychodromus acuminatus*, which analogically to *T. pyriformis*, is also able to quickly eliminate vaccinia virus from its nutrient medium, did not confirm this phenomenon. Electron microscopy of *O. acuminatus*, incubated with vaccinia virus (Jareño et al., 1985; Jareño, 1987), revealed that the virus was detectable only in the nutrient vacuoles of the protozoa, whereas the virions were observable within no more than 48 hrs. Almost at the same time with the appearance of virions into nutrient vacuoles some remarkable changes occurred also in the ultrastructure of the ciliates. Thus after one hour of incubation of *O. acuminatus* with vaccinia virus in the cytoplasm of the former, near mitochondria membranous cisterns (vesicles) could be seen. Their number grew constantly as far as virions were observable in nutrient vacuoles, but after disappearance of the latter also such formations vanished, eliminating thus differences in the ultrastructure of the ciliates. The authors explain that by hypertrophy of the Golgi apparatus of the ciliates, which occurred to eliminate substances released from vaccinia viruses which were being digested in nutrient vacuoles. The authors refer at that also to the possibility of the persistence of vaccinia virus in the Golgi apparatus, but we have failed to find in the publications of these authors any convincing data to confirm this hypothesis.

Nevertheless, quite persuasive data on the persistence and also replication of mammalian viruses in the organism of protozoa can be found in literature, of which we shall give a survey in the following part of the paper.

REFERENCES

- Bauer, L. Ein Beitrag zur Klärung der Beziehungen zwischen Amöben und Virus im Wasser // Arch. Hyg., Bact., 1961, 145, N 1, 12—20.
- Blawat, E., Kowalska, Z. Investigations in vitro on the influence of some amoeba on survival of poliomyelitis virus // Bull. Inst. Marine Med. in Gdansk, 1963, 14, N 1—2, 15—24.
- Chýle, M., Chýle, P., Stěpán, J., Patečka, T. Some enzymes and isoenzymes of *Trichomonas vaginalis* and changes in their activities after inoculation with a live mammalian virus // Casopis Čékau u Cesnkych, 1971, 110, 234—236.
- Chýle, M., Schneiderka, P., Stěpán, J. J., Chýle, P. Some enzyme and isoenzyme activities in *Trichomonas vaginalis* influenced by low temperature preservation and virus infection // Abstr. Int. Symp. Trichomonads and Trichomoniasis. Prague, 1985, 42.
- Groupé, V., Herrmann, E. C., Rausher, F. J. Ingestion and destruction of influenza virus by free-living ciliate *Tetrahymena pyriformis* (21625) // Proc. Soc. Exp. Biol. Med., 1955, 88, N 4, 479—482.
- Groupé, V., Pugh, L. Inactivation of influenza virus and of viral hemagglutinin by the ciliate *Tetrahymena geleii* // Science, 1952, 115, N 2986, 307—308.
- Jareño, M. A. Some ultrastructural changes produced by vaccinia virus in *Onychodromus accuminatus* (Ciliata, Hypotricha) // Acta Protozool., 1987, 26, N 1, 9—14.
- Jareño, M. A., Perez-Prieto, S., Garcia Gancedo, Y. A. Étude ultrastructurale préliminaire de quelques cilies inocules avec le virus de la vaccine // Protozool., 1980, 27, N 3, 83A.
- Jareño, M. A., Perez, S. F., Gancedo, A. The ciliates as useful tools in cytological studies of cell — virus interactions // Abstr. VII Int. Congr. Protozool. Nairobi, 1985, 30.
- Knorr, M., Klöhr, K., Kracher, H. Das Schicksal des Virus im Flusswasser und im Boden. II. Mitteilung: Virus und Amöben // Arch. Hyg., 1956, 140, N 1/2, 181—207.
- Perez-Prieto, S., Garcia-Gancedo, A. Interaction of *Tetrahymena pyriformis* and mammalian viruses // Abstr. III Intern. Congr. for Virology. Madrid, 1975, 249.
- Perez-Prieto, S. F., Garcia-Gancedo, A. G. Uptake of vaccinia virus by *Tetrahymena pyriformis* // Abstr. 4th Int. Congr. Virol. Hague, 1978, 557.
- Perez-Prieto, S., Garcia-Gancedo, A. La infección vírica de protozoos y las posibles implicaciones ecológicas y epidemiológicas. Estado actual del problema // Revista da sanidad e higiena publica, 1979, 53, N 11—12, 1517—1537.
- Perez-Prieto, S., Garcia-Gancedo, A. Uptake of vaccinia virus by *Tetrahymena pyriformis* // Microbiol. Exp., 1981, 34, 29—43.
- Perez, S., Garcia-Gancedo, A., Jareño, M., Vilas, P. On the experimental cocultures of *Tetrahymena pyriformis* with several RNA and DNA viruses // Abstr. VII Intern. Congr. Protozool. Nairobi, 1985, 133.
- Teras, J., Jögiste, A., Kallas, E. Theoretical aspects of the pathogenicity mechanisms of free-living protozoa // Abstr. II Intern. Congr. Parasitol. Munich, 1974, 1, 185.
- Teras, J., Kesa, L. The relationships between protozoa and viruses. 1. Protozoan viruses // Proc. Acad. Sci. ESSR. Biol., 1988a, 37, N 1, 17—26.
- Teras, J., Kesa, L. The relationships between protozoa and viruses. 2. Virus-like particles and cytopathogenic agent in protozoa // Proc. Acad. Sci. ESSR. Biol., 1988b, 37, N 3, 185—194.
- Терас Ю. Х., Кеса Л. Ю., Каллас Э. В. Экспериментальное исследование взаимоотношений *Tetrahymena pyriformis* с РНА- и ДНК-вирусами. IV. Об интеракции *T. pyriformis* с вирусом Coxsackie В-5 после контакта с *Myxovirus influenzae* типа А₂ // Взаимоотношения простейших с вирусами. Ч. 1. М., 1981, 126—136.

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ALGLOOMADE JA VIIRUSTE VAHEKORD

3. Algloomad kui viiruste inaktiveerijad

Peale endogeensete viiruste, viirusesarnaste partiklite ja viirusliku tsütopatogeense agensi uurimise algloomades (Teras, Kesa, 1988a, b) on paljud protozooloogid ja viroloogid püüdnud selgitada algloomade vahekorda ka imetajate viirustega. On tehtud kindlaks, et algloomad võivad imetajate viiruseid inaktiveerida (*resp.* elimineerida) ja et imetajate viirused võivad replitseeruda ja persisteeruda algloomade organismis.

Et algloomad võivad imetajate viiruseid inaktiveerida, tegid esmakordselt kindlaks V. Groupé ning L. Pugh (1952) *Tetrahymena pyriformis*'e akseenilises kultuuris, kuhu nad lisasid gripiviiruse A-tüübi (tüvi PR/8) või B-tüübiga (tüvi Lee) nakatatud kana-embrüo allantoisivedelikku. Niisama kiiresti kui gripiviirus kadus *T. pyriformis*'e kultuurist ka Newcastle'i viirus, kuid analoogilistes katsetes *Glaucoma scintillans*'i, *Euglena gracilis*'e ja *Astasia klebsi* kultuuridega jäi mõlema viiruse tiiter muutumatuks (Groupé jt., 1955).

Hiljem on kirjeldatud mitme viirustüübi inaktiveerimist ka *Naegleria* perekonda kuuluvate amööbiliikide poolt, samuti on korduvalt kinnitust leidnud gripiviiruse kiire elimineerimine *T. pyriformis*'e kultuuridest. Seda fenomeni on põhjalikult uurinud autorid, kes tegid kindlaks, et peaaegu niisama kiiresti kui inimese gripiviiruse tüüpe on *T. pyriformis* võimeline inaktiveerima (*resp.* elimineerima) linnu, sea ja hülge gripiviirust. Samuti õnnestus autoritel tõestada, et *T. pyriformis*'e võime gripiviirust inaktiveerida/elimineerida säilib ka algloomade korduvates passaažides viirust sisaldavates söötmetes, kuigi mõnes passaažis võivad algloomad sellest ajutiselt ka «keelduda».

Originaalseid tulemusi viiruste inaktiveerimise kohta algloomade poolt on oma uurintegutes saanud ka hispaania autorid, kes, kasutades mudelina *T. pyriformis*'e kõrval vabalt-elavat tsiliaati *Onychodromus acuminatus*'t, jälgisid samaaegselt viroloogiliste meetoditega elektronmikroskoopiliselt vaktsiini-, Sindbisi, vesikulaarse stomatiidi, herpesviiruste 1 ja 2, samuti enteroviiruste mitme tüübi saatust algloomade kultuurides.

Viiruseid inaktiveeriv/elimineeriv võime on seni sedastatud peamiselt vabaltelavate algloomade liikidel. Parasitaarsete liikide puhul võib kirjandusest leida andmeid vaid pseudorabiese viiruse inaktiveerimise kohta *Trichomonas vaginalis*'e poolt.

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ВЗАИМООТНОШЕНИЕ МЕЖДУ ПРОСТЕЙШИМИ И ВИРУСАМИ

3. Простейшие как инактиваторы вирусов

Кроме исследования эндогенных вирусов, вирусоподобных частиц и вирусного цитопатогенного агента в простейших многие протозоологи и вирусологи пытались выяснить и взаимоотношения простейших с вирусами млекопитающих. Установлено, что простейшие могут инактивировать (*resp.* элиминировать) вирусы млекопитающих и, кроме того, вирусы млекопитающих могут реплицироваться и персистировать в организме простейших.

Инактивация вирусов млекопитающих простейшими впервые была установлена в 1952 г. в аксенической культуре *Tetrahymena pyriformis*, в которую добавляли аллантоисную жидкость куриного эмбриона, зараженного вирусом гриппа типа А (штамм PR/8) или типа В (штамм Lee). Так же быстро исчезал из культуры *T. pyriformis* и вирус Newcastle, однако в аналогичных опытах с культурами *Glaucoma scintillans*, *Euglena gracilis* и *Astasia klebsi* титр обоих вирусов оставался неизменным.

Позднее описана инактивация разных типов вирусов некоторыми видами амёб семейства *Naegleria*. Феномен элиминации вируса гриппа из культуры *T. pyriformis* обстоятельно исследовали и авторы. Установлено, что *T. pyriformis* способен почти с такой же скоростью, как человеческие типы вируса гриппа инактивировать и птичий, свиной и тюлений типы данного вируса. Способность *T. pyriformis* инактивировать / элиминировать вирус гриппа сохраняется и при повторных пассажах простейших в вирусодержащих средах, хотя в некоторых пассажах простейшие могут от этого временно «отказываться».

Представляет интерес, что способность инактивировать / элиминировать вирусы до сих пор в основном установлена у видов свободноживущих простейших. Из паразитических видов в литературе имеются данные только об инактивации вируса псевдорabiesа со стороны *Trichomonas vaginalis*.