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ANTHROPOGENIC PRESSURE AND CHANGES IN *IXODES* TICK POPULATIONS IN THE BALTIC REGION OF RUSSIA AND DENMARK

АНТРОПОГЕННОЕ ВОЗДЕЙСТВИЕ И ИЗМЕНЕНИЯ В ПОПУЛЯЦИЯХ КЛЕЩЕЙ РОДА *IXODES* В БАЛТИЙСКОМ РЕГИОНЕ РОССИИ И ДАНИИ

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ABSTRACT

The representatives of two *Ixodes* species were tested for exoskeleton abnormalities and infections caused by different pathogens. Two groups comprising 3854 alive ticks from Russia and Denmark and 3417 alcohol fixed ticks collected from five different locations in 1990–1999 were studied.

Abnormalities were looked for using a stereomicroscope. For the first time the presence and types of *Ixodes* species nymphs' exoskeleton abnormalities were shown. Darkfield microscopy and IFA methods were used to detect infection.

The minimum of anomalies (10–15%) was found in protected areas: in the Zhiguli Reserve (Russia) and the Gribskov National Park (Denmark). The maximum (48%) was found in the industrial Cherepovets area (Russia). The prevalence of the exoskeleton abnormalities in *Ixodes* ticks populations depends greatly on the anthropogenic pressure. Exoskeleton abnormality research may be one of the most reliable ways of biomonitoring of the environment pollution level. Further research needed to reveal the exact abnormality-causing agents. It might be an accumulation of the heavy metal ions or complex hydrocarbons in the environment as well as any other factors.

РЕЗЮМЕ

С целью выявления аномалий экзоскелета и зараженности различными патогенами были изучены представители двух видов рода *Ixodes*. Исследовано 3854 живых клеща из России и Дании и 3417 клещей из спиртовых сборов в пяти различных регионах, собранных с 1990 по 1999 год.

Аномалии выявляли с помощью бинокулярного микроскопа. Впервые показано наличие и характер аномалий экзоскелета у нимф двух видов рода *Ixodes*. Для идентификации патогенов применяли темнопольную микроскопию и ИФА.

Минимальное число аномальных клещей (10–15%) было обнаружено в сборах из охраняемых природных территорий: Жигулевского заповедника (Россия) и Национального парка Грибсков (Дания), максимальное (48%) — в окрестностях Череповецкой индустриальной зоны (Россия). Встречаемость аномалий экзоскелета в популяции иксодовых клещей напрямую зависит от степени загрязненности окружающей среды. Исследование аномалий может служить одним из возможных способов биомониторинга состояния среды. Непосредственные причины возникновения аномалий нуждаются в дальнейшем исследовании. Ими могут быть накопление в среде тяжелых металлов, сложных углеводородов и другие факторы.

INTRODUCTION

The abnormalities in the Ixodoidea were studied for a long time. Previous authors most often described the very rare abnormalities, such as the hynandromorphism [Pavlovsky, 1939] or the gnathosoma absence [Koshkin, 1967]. First classifications of the abnormalities in the Ixodoidea were made by Pavlovsky [1939], Pervomaisky [1954] and Campana-Rouget [1959]. They remarked a very small quantity of anomalies (less than 1%). They suggested that the interspecific cross-breeding or some traumatically or chemically-induced influences during the preimaginal period of the tick development would lead to developing the abnormalities.

Earlier several successful experiments on the artificial terathogenesis in Acarina were done. The ethylquinone was used to induce the abnormalities in the Ixodoidea experimentally [Campana-Rouget, 1959]. The exoskeleton abnormalities in the Oppioidea were produced by adding the nickel ions into the mite diet [Dubinina, Alekseev, 1994].

We examined the anthropogenic pollution influences on *Ixodes persulcatus* Schulze and *Ixodes ricinus* L. ticks, the vectors of important diseases such as tick-borne encephalitis (TBE), Lyme disease, and other tick-born borrelioses and ehrlichioses. The first recorded pollution influence was described earlier as the appearance of the exoskeleton abnormalities [Alekseev, Dubinina, 1993; Alekseev, 1995] and various tick reactions on the *Borrelia* infection [Alekseev, Dubinina, 1997].

This research is an attempt to find out if the anthropogenic pollution can cause in the exoskeleton abnormalities in ticks.

MATERIALS AND METHOD

Ticks from different areas of Russia and Denmark with extremely varied pollution levels were studied to determine the anthropogenic pressure influences.

The ticks *I. persulcatus* were collected 1993–1999 in the recreational zone of St. Petersburg, Russia, on the North-East beach of the Finnish Gulf. More than 2500 samples were taken. This region was registered as a focus of the TBE, the tick-borne borreliosis infection and is suspected for the ehrlichiosis.

In Denmark *I. ricinus* ticks were collected in two places: in the National Park Gribskov (North Zealand, 80 km from Copenhagen) and the recreational zone of Kongelunden near the Kastrup International Airport, Copenhagen in 1999 (471 samples taken).

Apart from this, a large collection of *I. ricinus* ticks (2946 specimens) previously collected in Zhiguli National Preserve (Russia) in 1990 and fixed in 70% ethanol was also investigated.

Alive *I. persulcatus* ticks were collected by flagging and transferred to the laboratory. Their locomotor activity was measured with the help of original methods by Alekseev [Alekseev, 1996; Alekseev et al., 1996; Alekseev et al., 2000].

All collected ticks were examined with a stereomicroscope to detect exoskeleton abnormalities. Anomalous *I. ricinus* adults were taken under the stereomicroscope and scanning electronic microscope (SEM).

All alive ticks collected in Russia were examined for TBE and borreliosis infection by IFA analysis using the polyclonal antibodies and fluorescent microscopy. The facts of *Borrelia* presence and infection rate intensity among collected ticks were estimated using darkfield microscopy (DF) for alive spirochetes. By the use of PCR and RLB methods DF positive specimens were studied for the identification of *Borrelia* species. Ticks collected in Denmark were examined for the exoskeleton anomalies only.

RESULTS AND DISCUSSION

The research yielded a large number of different exoskeleton abnormalities. The abnormalities in the adult *I. persulcatus* were described earlier [Alekseev, Dubinina, 1993, 1997; Alekseev, 1995, 1996]. Since that time we have succeeded in demonstrating the fact that similar exoskeleton abnormalities can be found in the adult *I. ricinus* and also in *I. ricinus* and *I. persulcatus* nymphs in Denmark. The number of anomalous nymphs detected in all investigated populations was less than 1%. The main type (more then 95%) of abnormalities found is the various deformations of the scutum. Different types of scutum deformations are shown in Fig. 1–6. The rare abnormalities such as the gnathosoma deformations are presented in Fig. 7–8.

Despite the fact that anomalous ticks are very different in their outward appearance, they can be placed in one conditional group. This group will differ from normal ticks not only morphologically by the presence of anomalies, but also by several other distinct features.

The locomotor activity of anomalous ticks of both species of *Ixodes* is significantly lower in comparison with the normal ones [Alekseev, Dubinina, 1997; Alekseev et al., 2000]. Vice versa, if the tick is infected the locomotor activity of normal ticks decreases whereas it shows the great increase in anomalous ticks (Fig. 9).

Different numbers of anomalous ticks were detected in areas affected by different amount of pollutants (Fig. 10). The maximum (48%) was detected in the population of *I.persulcatus* near the city of Cherepovets (Russia), where the major metallurgical integrated plant is situated [Alekseev, 1995].

The next two findings are related to St. Petersburg, where studies were accomplished in 1993–1999. The collection place is situated near the heavy traffic highway and is exposed to the influence of aerosol wastes from St. Petersburg's North-East industrial complex. The recorded decrease in the number of abnormalities, from 41% in 1993–1995 to 32% in 1996–1999, may be explained by the decrease in the industrial production in St.Petersburg during that period.

In Denmark the largest amount of abnormalities (23%) was registered in the area, which is very closely located to the Kastrup International Airport in Copenhagen. In preserved areas such as

Fig. 1. Widely distributed deep scutum deformation. × 40. *I.ricinus*, female. 1999, Denmark.
Рис.1. Широко распространенный тип деформации скутума. × 40. Самка *I. ricinus.* 1999, Дания.

Fig. 2. The deeply lined hinder part of the scutum. × 35. *I.ricinus*, female. 1999, Denmark.
Рис. 2. Складчатая нижняя часть скутума. × 35. Самка *I.ricinus.* 1999, Дания.

Fig. 3. Multiple chaotic depressions on the scutum. × 45. *I.ricinus*, male. 1999, Denmark.
Рис. 3. Множественные хаотичные вдавления на скутуме. × 45. Самец *I.ricinus.* 1999, Дания.

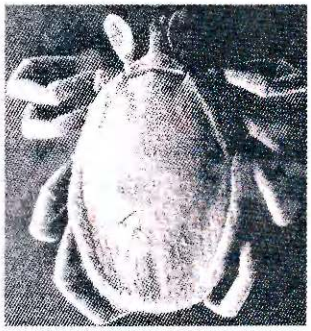

Fig. 4. Deep depression of the hinder part of the scutum. × 40. *I.ricinus*, female. 1999, Denmark.
Рис. 4. Глубокая вдавленность в нижней части скутума. × 40. Самка *I.ricinus.* 1999, Дания.

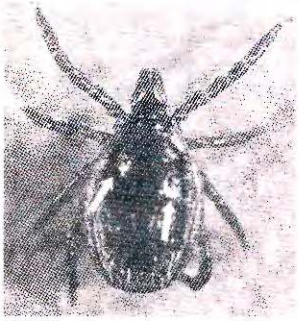

Fig. 5. Scutum deformations. × 55. *I.ricinus*, nymph. 1999, Denmark.
Рис. 5. Деформации скутума. × 55. Нимфа *I.ricinus.* 1999, Дания.

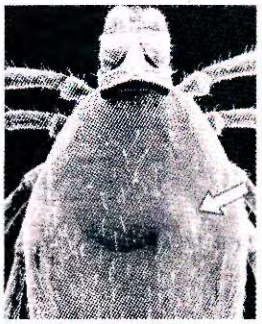

Fig. 6. Asymmetrical deformation of the right hinder part of the scutum. × 40. *I.ricinus*, female. 1999, Denmark.
Рис. 6. Ассиметричная деформация нижней правой части скутума. × 40. Самка *I.ricinus.* 1999, Дания.

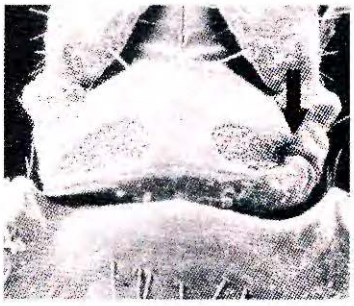

Fig. 7. Anomalous deep fissure of the basis capituli right side. × 100. *I.ricinus*, female. 1999, Denmark.
Рис. 7. Глубокая аномальная щель на правой стороне основания гнатосомы. × 100. Самка *I.ricinus.* 1999, Дания.

Fig. 8. Reduced right palp. × 100. *I.ricinus*, female. 1999, Denmark.
Рис. 8. Укороченная правая пальпа. × 100. Самка *I.ricinus.* 1999, Дания.

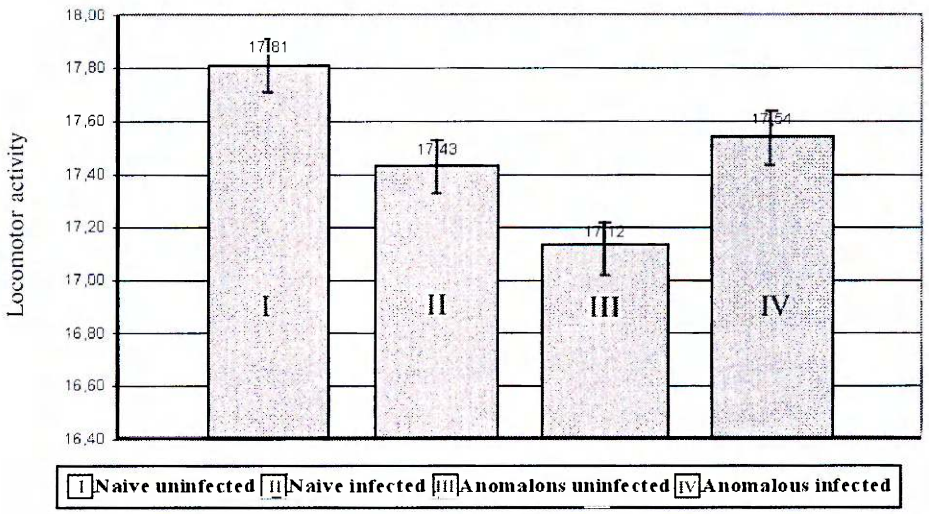

Fig. 9. Suppression of locomotor activity of normal and anomalous *Ixodes persulcatus* ticks depending on *Borrelia* infection (1995–1999).

Рис. 9. Подавление двигательной активности нормальных и аномальных клещей *Ixodes persulcatus* в зависимости от зараженности боррелиями (1995–1999).

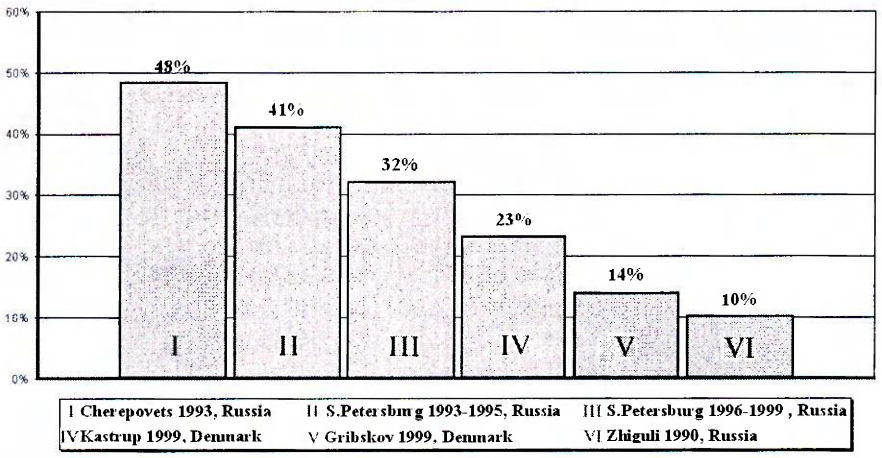

Fig. 10. Occurence of anomalous *Ixodes* ticks collected in different areas.

Рис. 10. Встречаемость аномалий у клещей рода *Ixodes*, собранных в различных регионах.

the Gribskov National Park (Denmark) and the Zhiguli National Reserve (Russia) the smallest number of abnormalities was recorded (less than 15%).

There is a noticeable linear dependence between the number of anomalous ticks in a population and the anthropogenic pollution level.

CONCLUSION

Ixodes ricinus represents the same types of the exoskeleton anomalies as *I.persulcatus*. Nymphs are a subject to the formation of abnormalities, which aresimilar to adult ticks. This phenomenon is reported for the first time.

Anomalous ticks differ from normal ones by many parameters, including various exoskeleton abnormalities and the lower locomotor activity.

The number of anomalous ticks in a population greatly depends on the anthropogenic pressure. It is high in the areas with the higher pollution level.

Exoskeleton anomaly research can be a reliable method of biomonitoring of the environment pollution level.

The exact terathogenesis-causing agent is not known. Further investigations are necessary.

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