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Biochemical Characteristics of Human and Animal Disease Carriers Acari Parasitiformes.

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ABSTRACT

During the study of the supernatants homogenates among Ixode, relapsing-fever blood-sucking ticks belonging to five different genera and ten species in polyacrylamide gel by electrophoresis the species differences were revealed in electrophoretic mobility of the tick protein fractions. Relapsing-fever ticks demonstrate as generic so as specific differences during the nymphal stage of development. The nymphs 3 Ornithodoros papillipes contain two protein fractions, O.moubata contain 3 fractions and Alveonasus lahorensis contain 5 fractions. The same kind of ticks had the same amount of protein fractions at the electrophoretic separation of proteins. During the larval stage the homogenates of ticks always show one protein fraction. The hemolymph of A.lahorensis tick showed the differences between imago and nymph 3. The antibacterial activity of the supernatant homogenate mite is revealed. The correlation between the value of antibacterial activity of the supernatant homogenate of ticks and the amount of protein fraction is showed at the electrophoresis in polyacrylamide gel. A new biochemical marker is proposed to state the species of ticks.

Keywords: Electrophoretic study of Alveonasus lahorensis, Ixodes ricinus ticks.

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INTRODUCTION

In recent years, it became necessary to study biochemical blood-sucking arthropods of a body (ticks, fleas, mosquitoes) due to the identification of their specific role in the transmission and long-term preservation of various human disease agents.

The study of biological relationship issue between arthropods and pathogens, the establishment of species differences in the relationship with an agent require precise biochemical characteristics of different bloodsuckers as the environment which may store and develop a pathogen for a long time. A number of researchers showed that a carrier's body is a complex living system, but not an inert habitat for any pathogen. A Pathogen and a carrier influence each other and this is reflected in the metabolism of a carrier and of a pathogen [1-5]. One may believe that the study of differences in tissues and organs of arthropods that have medical significance, will provide new information about the features of the hemolymph protein composition, about the biochemical differences of different species, and to get closer to the study of relationship problem between carriers and agents.

TICK PREPARATIONS FOR ELECTROPHORESIS.

Preparations for electrophoresis were prepared as follows: homogenates were prepared from ticks. Hemolymph was taken from the leg and was added in a small volume container with 0.85% of saline solution or 100 mcl of phosphate-buffer saline and sonicated three times using MZE device (England) at maximum power for 10 seconds at 4 °C and centrifuged for 5 min at 5000 rev/min to remove insoluble material. The resulting supernatants determined the protein content by Lowry, added an equal volume of lysing buffer of the following composition: 8% of dodecyl sulfate Na, 30% of glycine, 5% of 2-mercaptoethanol in 0.25 M Tris - HCL buffer, pH 6.8. The lysates were heated at 100 C for one minute and were processed by disc-electro-phoresis in the block of 15% polyacrylamide gel with the thickness of 1 mm., using an alkaline system. The separation continued until the bromophenol blue marker dye reached the lower boundary of the gel. The current intensity was 20 mA during the first 30 minutes and 40 mA, - after entry of the samples in the separating gel. Gels were stained with 0.04% solution of Coomassie G - 250 in 4% chloric acid for 30 minutes. Gels were washed in 5% acetic acid [5].

TICKS UNDER STUDY

We used the following relapsing-fever ticks in our work: p. Alveonasus, Ornithodoros (A.lahorensis, O.papillipes and O.moubata).

X-shaped ticks were represented by 3 types: Dermacentor, Hyalomma and Ixodes (D.andersoni, D.nuttalli, H.asiaticum, H.dromedarii, H.anatolicum, I.persulcatus, I.ricinus). Micrococcus luteus ATCC 4698 was used as a culture. In the future, the study of electrophoregrams was performed. The supernatant for biochemical studies was used from 10 experiments.

OBTAINED RESULTS DISCUSSION

Fig. 1 shows an electrophoretograms of homogenate proteins from ticks of different families, genera and species. For the convenience of an electrophoretogram consideration the female and the male homogenates of one species of ticks were located closely.

When the homogenate of this tick nais was studied, its electrophoretogram was also located nearby. We compared the homogenates of 2 genera by electrophoresis among argasidae ticks: Alveonasus and Ornithodoros. The electrophoregrams of A.lahorensis tick homogenates have 3 protein fractions among females and males and 5 fractions among nais. The homogenates of nais 3 among O.papillipes tick contain 2 protein fractions and the homogenate of nais 3 O.moubata contains 3 protein fractions.

Thus, 3 species of studied ticks A.lahorensis, O.papillipes and O.moubata have a marked resemblance in electrophoretic separation of proteins in the homogenates of adult females and a clear difference in the electrophoretic separation of proteins in the homogenates of ticks during the nymphal stage of development.

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The X-shaped ticks of all 3 species among Hyalomma genus (H.asiaticum, H.dromedarii, H.anatolicum) had a similar electrophoretic separation of homogenate proteins. The intensity stripes made the only difference, but this difference may be due to different concentration of protein in homogenates. These ticks clearly showed 3 protein fractions, and the 1st one was slow, and the 2nd and the 3rd one was more mobile, moving within a close distance from each other. By its electrophoretic pattern the genus Dermacentor was different from the genus Hyalomma. The homogenates of D.andersoni ticks revealed 5 protein fractions and the homogenates of D.nuttalli ticks had 4 protein fractions. We were unable to identify clear differences in the electrophoretic separation of proteins among females and males.

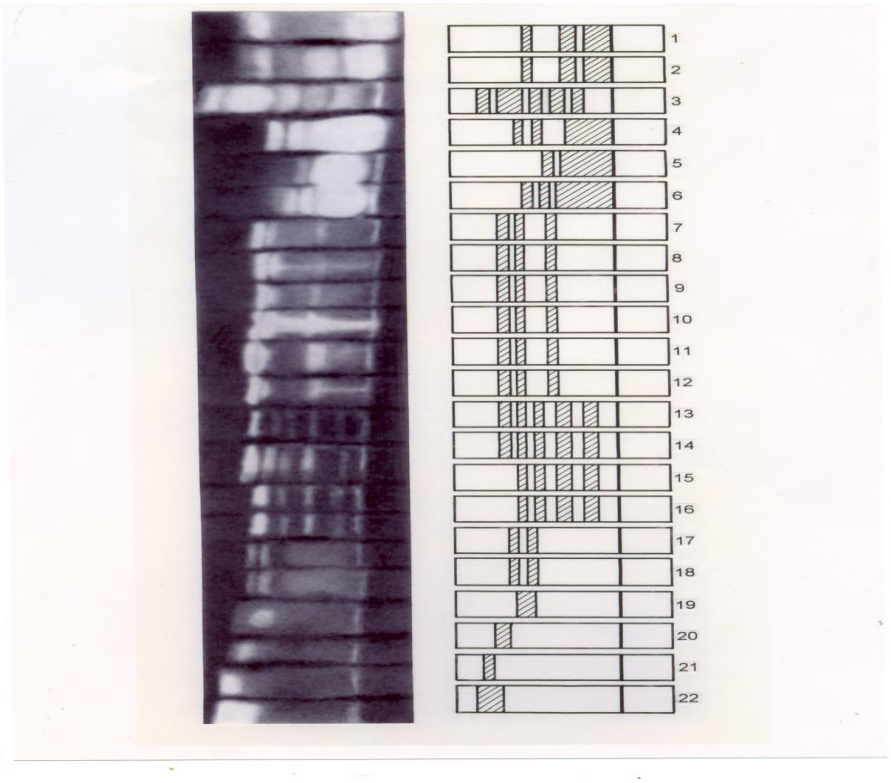

Figure 1: Electrophoregram of tick homogenates in polyacrylamide gel.

1. Alveonasus lahorensis (females)
2. A.lahorensis (males)
3. A.lahorensis (nais 3)
4. Ornithodoros papillipes (females)
5. O.papillipes (nais 3)
6. O.moubata (females)
7. Hyalomma asiaticum (females)
8. H.asiaticum (males)
9. H.dromedarii (females)
10. H.dromedarii (males)
11. H.anatolicum (females)
12. H.anatolicum (males)
13. Dermacentor andersoni (females)
14. D.andersoni (males)
15. D.nuttalli (females)
16. D.nuttalli (males)
17. Ixodes persulcatus (adults)
18. I.ricinus (adults)
19. I.persulcatus (larvae)
20. I.ricinus (larvae)
21. O.papillipes (larvae)
22. O.moubata (larvae).

The representatives of Ixodes genus ticks (I.persulcatus, I.ricinus) revealed a completely different electrophoretic pattern than the ticks of the genus Hyalomma and Dermacentor. 2 clearly protein fractions were revealed, and the larvae had only one faction.

Considering the findings as a whole, obvious differences in protein composition of tick homogenates between two families and multiple genera should be noted. However, the species differences were not always established within the same genus. On the contrary, some common elements were revealed in the electrophoretic pattern of protein separation in homogenates within the same kind of ticks (during imago phase). There are clear differences among genera and species during the nymphal stage of ticks development. The homogenates of ticks during the larval stage of development always reveal no more than one protein

fraction. Perhaps the process of protein differentiation during the larval phase is expressed weakly. It is also possible that the applied methods of protein extraction and distillation were not allowed to identify all protein fractions of the larvae.

Figure 2 shows electrophoretograms that are typical for A.lahorensis tick hemolymph. As Fig. 2 shows A.lahorensis tick hemolymph contains clearly visible protein fractions. One of these fractions is less mobile, and three fractions are more mobile. The comparison of hemolymph and pure bovine albumin electrophoregrams shows that in the hemolymph of A.lahorensis ticks the fast moving fractions are somewhat behind the albumin in respect of its electrophoretic mobility.

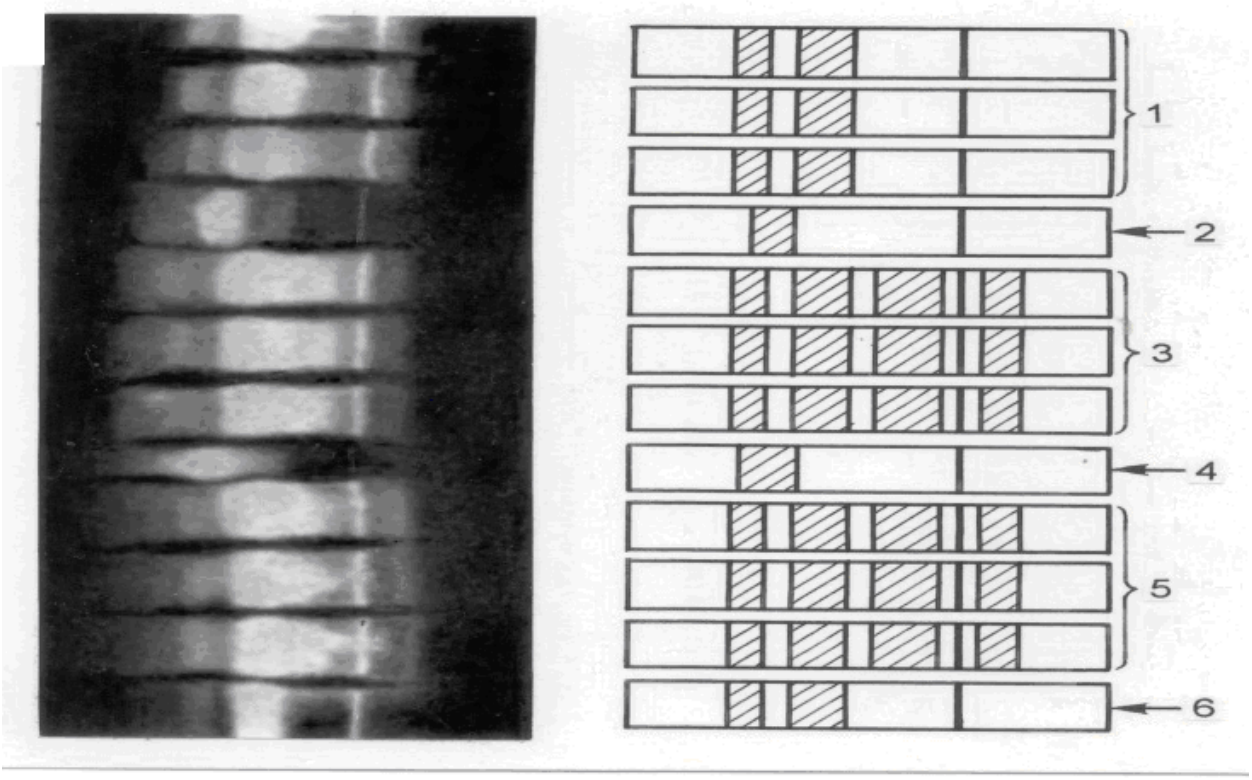

Figure 2: Electrophoregram of Alveonasus lahorensis tick hemolymph.

1. Nymphs 3 of A.lahorensis.
2. The larvae of A.lahorensis
3. A.lahorensis females
4. Bovine albumin
5. A.lahorensis males
6. Guinea pig serum.

The consideration of A.lahorensis nymph hemolymph electrophoretogram shows that the tick hemolymph during the nymphal stage has somewhat different electrophoretic pattern than that of imago. The nymph hemolymph does not contain the least mobile fraction which is observed among adult males and females, and the fraction following it is a more compact one. We may assume that the electrophoretic mobility of hemolymph proteins amog the ticks A.lahorensis changes in the development, as evidenced by marked differences in the electrophoretic pattern between imago and nymph 3. The electropherograms of bovine serum were obtained under the same conditions for comparison. According to the electrophoretic mobility the proteins of tick homogenates are weaker than serum albumin and are more in line with G - globulins. Concerning the issue of sex differences in the electrophoretic pattern of male and female proteins: our experiments did not reveal significant sex differences in the proteins of A.lahorensis tick.

According to Fig. 3, the highest antibacterial effect was detected in the supernatant of homogenates among A.lahorensis ticks, containing large amounts of protein fractions (Figure 3 - № 3 and 5). Less pronounced antibacterial action was detected in the supernatant of I.ricinus tick homogenates. A small effect was shown by supernatant homogenate of I.ricinus tick larvae.

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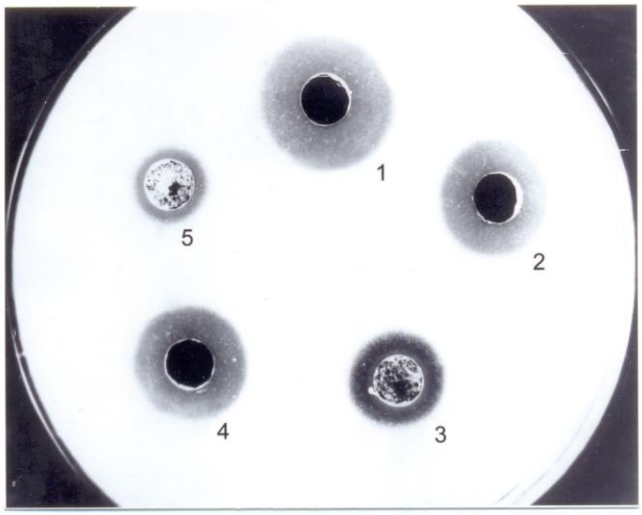

Figure 3: The antibacterial effect of Alveonasus lahorensis, Ixodes ricinus ticks supernatants homogenates concerning Micrococcus luteus ATCC 4698.

1. Egg lysozyme 8 mg / ml.
2. The homogenate of A.lahorensis ticks.
3. The homogenate of I.ricinus ticks.
4. The homogenate of A.lahorensis ticks.
5. The larvae of I.ricinus ticks.

SUMMARY

Based on the abovestated facts we may conclude that during the study by electrophoresis method the polyacrylamide gel of the supernatants homogenates of argasid ticks and the ticks belonging to five different genera and 10 species specific differences in the electrophoretic mobility of the tick protein fractions were revealed. Argasids showed generic and specific differences during the nymphal stage. The nymphs 3 O.papillipes contained 2 protein fractions, O.moubata contained 3 fractions and A.lahorensis contained 5 fractions. The same number of protein fractions was observed among one genus of ticks during the electrophoretic separation of proteins. A protein fraction of homogenates ticks was always revealed during the larval stage. The hemolymph of A.lahorensis tick revealed the differences between imago and nymph 3. An antibacterial activity of the supernatant homogenates of ticks was determined. The correlation between the antibacterial activity of the tick supernatant homogenate and the quantity of protein fractions is shown during the electrophoresis in polyacryl-amide gel. A new biochemical marker is proposed to determine a species of ticks.

CONCLUSION

Thus, the study of various supernatants of homogenates of different tick species revealed differences in antimicrobial activity. The obtained results are correlated with the values of protein fractions of the obtained species of ticks and may serve as biochemical markers to determine a species of ticks.

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