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ACQUIRED RESISTANCE OF PIGEONS TO ARGAS POLONICUS LARVAE

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Abstract. The acquired resistance of pigeons to *Argas polonicus* manifests itself in an adverse effect on the feeding and further development of larvae. After five subsequent infestations, the rejection of larvae reached 90—95 % and the mean weight of fed larvae dropped down from 2.18 mg to 1.72 mg in the Polish tick population. There appeared also a tendency to more rapid feeding in larvae from later infestations. Also the percentage of moulted larvae decreased, probably as a result of insufficient blood meal taken by larvae on repeatedly challenged hosts. The hosts sensitized by larvae of Polish tick population manifested a higher tick resistance than those sensitized by larvae of Czechoslovak population. No adverse effect has been observed in males and females after repeated infestation of sensitized pigeons. Anti-tick antibodies were detected in the serum of pigeons sensitized by larvae with the aid of counter current immunoelectrophoresis using extracts from whole larvae and from salivary glands of adults as antigen. No antibodies were detected in the serum of pigeons sensitized by adult ticks.

The acquired resistance to ticks has been intensively studied all over the world (Willadsen 1980, McGovan and Barker 1980, Wikel 1982, Wikel and Allen 1982, Oberem 1984, Wikel 1984, Brown 1985, Wikel and Whelen 1986 and others). However, relatively few papers deal with the acquired immunoresistance of hosts to argasid ticks. Attention has been paid to the rejection of larval *Ornithodoros venezuelensis* feeding on quinea pigs (Trager 1940), changes in the blood basophil levels, and to the cutaneous basophil hypersensitivity reaction occurring at the attachment sites of nymphal or adult *Ornithodoros tartakovskyi* feeding on guinea pigs (Askenase and Worms 1979, Brown et al. 1983, McLaren et al. 1983) or of *Ornithodoros parkeri* and *O. moubata* feeding on rabbits (Centurier et al. 1981, Johnston and Brown 1985). Shatrov (1979) observed pathological changes in the rabbit skin during feeding of *Alveonasus lahorensis*. Brossard et al. (1981) detected the presence of antibodies against salivary gland tissues of *Ornithodoros moubata* in the serum of a sensitized rabbit by indirect immunofluorescence method and observed also a little adverse effect on the adult tick biology. The blood intake was found to be sometimes smaller and the feeding period somewhat longer. The attachment to the host and oviposition were not adversely affected. On the other hand, no adverse effect was observed by Centurier et al. (1981) in nymphs of *Ornithodoros moubata* when feeding on repeatedly infested rabbits, although intensive antibody formation was demonstrated by means of ELISA.

A single attempt to evaluate the problem of acquired immunity to argasid ticks in bird hosts is described in the paper by Trager (1940). However, the results of his experiments with chickens and *Argas persicus* were not satisfactory and did not demonstrate the existence of acquired resistance of chickens to nymphs, adults and even larvae of *A. persicus* (see also Brown 1985). The skin reaction of naive host chickens to feeding of *Argas persicus* larvae was studied by Moorhouse (1975). The author observed a great accumulation of heterophil leukocytes at the feeding site.

Since a rejection of larvae appeared in the secondary and later feedings in our experiments with *Argas polonicus* larvae repeatedly fed on pigeons, we have dealt with this problem more deeply.

MATERIALS AND METHODS

Tick populations of the species *Argas* (*Argas*) *polonicus* Siuda, Hoogstraal, Clifford et Wassef, 1979 bred at the Institute of Parasitology, Czechoslovak Academy of Sciences, were used in our experiments. The ticks originated from the nesting place of pigeons in the loft of the St. Michael Chapel at Košice, Czechoslovakia (28 April 1981) and from the type locality of the species in St. Mary's Church at Krakow, Poland (1 June 1981 and 17 September 1982). The ticks were kept for some generations in the laboratory at the temperature of 27 ± 1 °C and 75 ± 5 % RH in darkness.

Host pigeons were either obtained from breeders or wild pigeons were captured in the town outside the distribution area of *A. polonicus*. Five pigeons were infested with 100—200 larvae of Polish population and three pigeons by larvae of Czechoslovak population at the age of 18—90 days at two-week intervals (17 infestations altogether). Three other pigeons were infested by 10 adult ticks (5♀ and 5♂ — 9 infestations altogether) at weekly intervals. The number of successfully engorged larvae, total weight of engorged larvae detached from the host on individual days, percentage of moulted larvae in individual infestations, differences in the weight of groups of adults before and after feeding, duration of preoviposition and preeclosion periods, number of eggs deposited, and percentage of larvae hatched from eggs of females from individual infestations were recorded. Engorged imagoes were kept separately by pairs, engorged larvae were kept together and checked twice a week. At the time of oviposition, egg hatching and moulting of larvae, the control was made daily.

Anti-tick antibodies in the serum of pigeons after the fifth infestation of larvae or nineth infestation of adults of the Polish population were detected by counter current immunoelectrophoresis using a whole larval extract and salivary gland extract from unengorged adults as antigens. Salivary glands of 20 adults or 50 larvae were homogenized in 0.1 M barbiturate buffer, pH 8.6. The homogenates were centrifuged at 4,000 g at 4 °C for 10 min. The supernatant with the resulting protein concentration of 0.15—0.20 mg . ml^{-1} (after Lowry et al. 1951) was used for the detection of antibodies in the serum of immunized pigeons by means of counter current immunoelectrophoresis in 0.8 % agarose gel at 10 V . cm^{-2} constant voltage in 0.1 M barbiturate buffer at pH 8.6 for 1 h. After termination of electrophoresis the gels were incubated in saline (pH 7.4) at 4 °C, washed in distilled water, dried, stained by Ponceau S, and the background was decolourized by 2 % acetic acid.

The results of experiments were evaluated in graphs, the parameters were estimated by Newton—Raphson's method on HP 9845A computer. Statistical significance of the detected differences was tested by Student's t-test and χ^2 (chi-square) calculation.

RESULTS

Repeated infestations of experimental pigeons by *A. polonicus* larvae induced a distinct immunoresistance of hosts affecting the larval feeding success. As evident from Fig. 1A and B, the percentage of larvae capable of a successful termination of feeding gradually decreased, so that after the fifth infestation its mean value was lower than 10 %. The initial feeding success in general was higher and the rejection of larvae

Table 1. Number of engorged larvae of Polish population detached from hosts during subsequent experimental infestations

No. of infestation	No. of engorged larvae / day of detachment						Total No. of engorged larvae	Total No. of larvae put on hosts
	4th day	5th day	6th day	7th day	8th day	9th day		
1	1	87	307	115	36	3	549	690
2	3	87	115	26	10	4	245	710
3		109	70	12	2		193	804
4	2	32	5	1			40	600
5	24	63	3	1			91	300
6	16	12	3	1			32	440
7	2	5	1				8	200

after repeated infestation was more marked in the larvae of Polish population. The differences in the number of successfully engorged larvae during the first five infestations were statistically highly significant (P < 0.01).

There was also a significant shift in the speed of larval feeding in the course of gradual immunization of hosts. Whereas during the initial infestations the majority of larvae

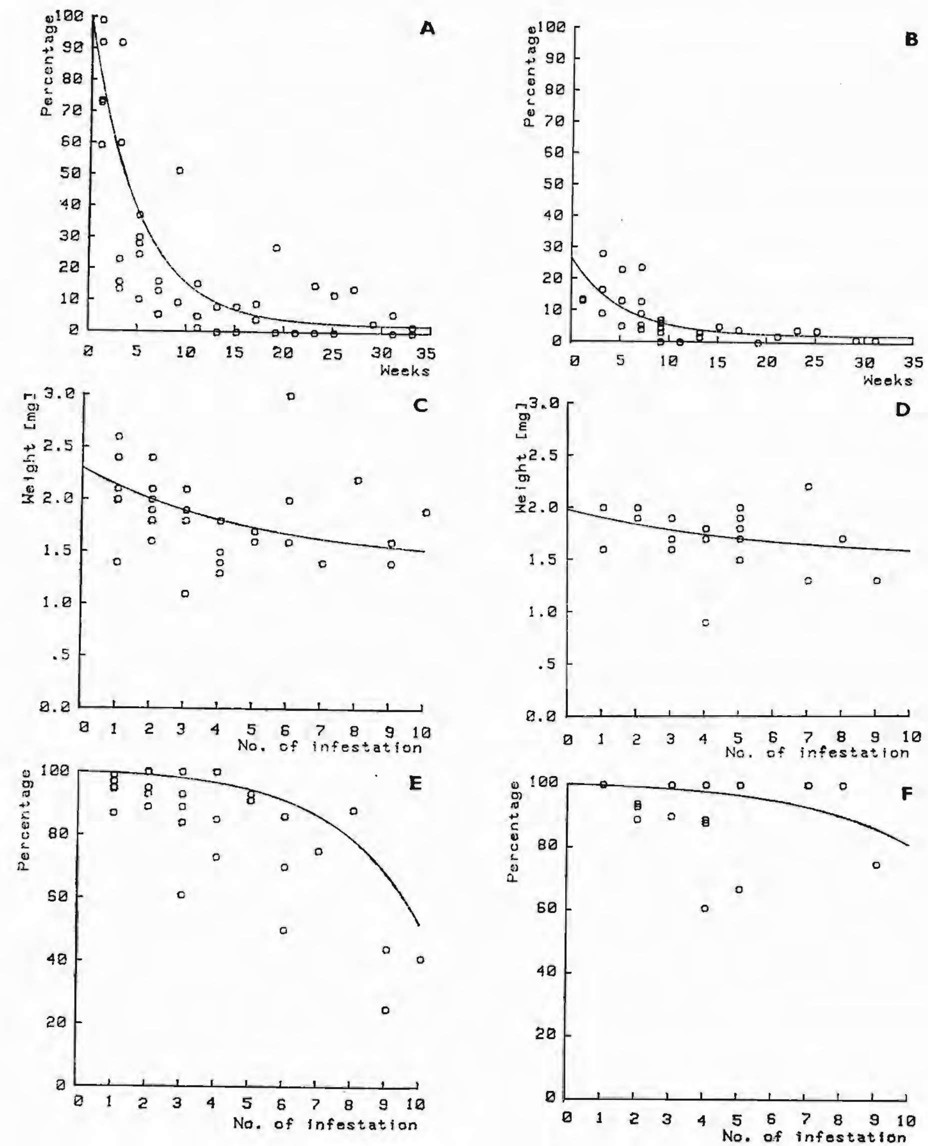

Fig. 1. Influence of subsequent experimental infestations of pigeons on the larvae of *Argas polonicus*. A — Percentage of succesfully engorged larvae of Polish population, B — and of Czechoslovak population, C — Mean weigt of engorged larvae of Polish population, D — and of Czechoslovak population, E — Percentage of moulted larvae of Polish population, F — and of Czechoslovak population.

detached on days 6 and 7 after infestation and the feeding lasted up to the 9th day, in the following infestations the majority of larvae detached on days 4 and 5 p.i. and all larvae terminated feeding already on day 6 or 7 p.i. However, this effect occurred only in the larvae of the Polish population (Table 1), whereas most of the larvae of the Czechoslovak population detached from the host on day 5 p.i. already in the initial infestations and all larvae terminated feeding not later than on day 6 or 7 p.i.

As to the mean weight of engorged larvae in individual infestations (Fig. 1C and D) in the Polish population the amount of accepted blood decreased with increasing number of infestations on the challenged pigeons. The differences in the mean weights of larvae in the first four infestations were statistically highly significant ($P < 0.01$). The highest weights in the first infestations were reached by the larvae detached on days 5—7 of feeding, in the following infestations, the larvae detached on days 4 and 5 were the heaviest (Table 2). In the larvae of the Czechoslovak population, this tendency was not very marked and the differences were mostly statistically insignificant ($P > 0.05$).

The percentage of moulted larvae slightly decreased with the increasing number of repeated infestations (Fig. 1E and F), but due to the low number of engorged larvae from the later infestations, it was statistically insignificant ($P > 0.05$).

Table 2. Mean weight [mg] of engorged larvae of Polish population dropping from the hosts during subsequent experimental infestations

No. of infestation	Mean weight [mg] / day of detachment						Total mean weight
	4th day	5th day	6th day	7th day	8th day	9th day	
1	1.80	2.12	2.39	1.92	1.53	1.60	2.18
2	1.45	1.73	2.28	1.99	1.20	- -	1.99
3		1.57	1.81	1.73	1.45		1.67
4	0.90	1.68	1.19	---			1.58
5	1.67	1.76	1.50	----			1.72
6	1.81	1.62	—	- -			1.73
7	1.00	1.60	1.00				1.38

Table 3. Mean amount of blood meal accepted by males and females, length of preoviposition and preeclosion periods and percentage of eggs hatching during and after subsequent experimental infestations [$\bar{x} \pm SD$]

No. of infestation	Mean amount of blood meal accepted by male [mg]	Mean amount of blood meal accepted by female [mg]	Preoviposition period [days]	Preeclosion period [days]	Eggs hatching [%]
1	17.0 ± 1.0	42.2 ± 1.3	65.5 ± 53.6	18.6 ± 4.6	100.0
2	19.2 ± 2.1	45.4 ± 5.3	78.2 ± 62.0	18.0 ± 6.0	99.7
3	17.8 ± 1.6	45.0 ± 3.1	67.0 ± 58.5	18.1 ± 4.8	95.5
4	19.8 ± 0.3	45.2 ± 1.9	66.3 ± 49.4	15.9 ± 5.2	98.5
5	16.9 ± 0.9	39.9 ± 10.3	82.8 ± 47.1	18.4 ± 6.8	100.0
6	18.3 ± 3.3	45.4 ± 1.3	87.9 ± 37.7	17.9 ± 3.9	94.5
7	16.5 ± 0.4	41.1 ± 2.2	91.1 ± 15.2	23.8 ± 5.4	94.5
8	17.1 ± 1.2	40.5 ± 0.8	95.5 ± 31.8	18.5 ± 3.2	99.3
9	17.8 ± 0.8	39.3 ± 7.6	80.9 ± 8.1	17.0 ± 3.1	100.0

The effect of a repeated feeding of males and females of the Polish population on the appearance of immunoresistance in pigeons was not demonstrated. No statistically significant differences ($P > 0.05$) were detected in the mean amount of blood accepted by a male or female during individual feedings on the challenged hosts, as well as in the duration of the preeclosion period in repeated infestations (Table 3). There appeared a slight tendency to prolong the preoviposition period in later infestations, but neither these differences were statistically significant ($P > 0.05$), due to the great dispersion. The number of eggs deposited was directly proportional to the mean weight of engorged females and this relation remained linear during the whole experiment (Fig. 2). The percentage of egg hatching was not affected by the repeated feeding as well (Table 3).

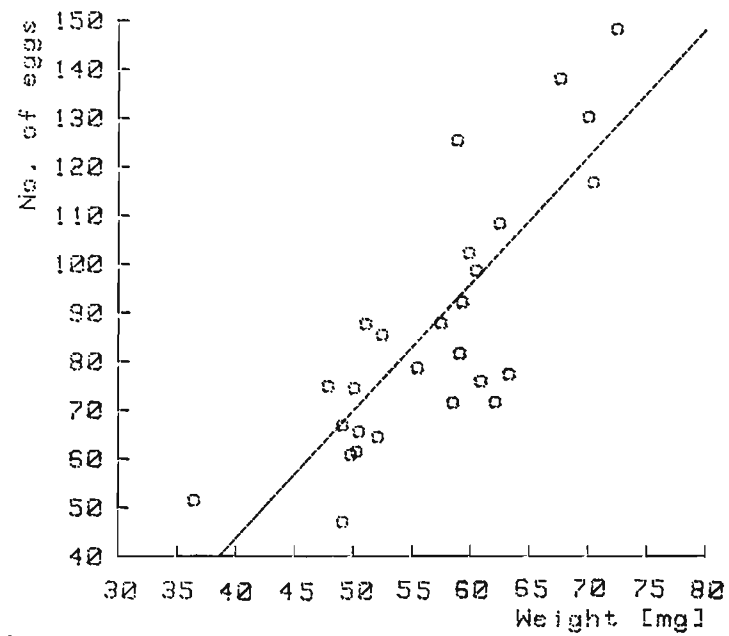

Fig. 2. Regression line of the dependence of egg number on engorged female weight (regression coefficient = 0.809).

Anti-tick antibodies could be detected by counter current immunoelectrophoresis only in the serum of pigeons sensitized by larvae. This serum reacted positively with the antigen from larval homogenate still in the dilution of 1 : 64 and with the antigen from salivary glands of adults in the dilution of 1 : 16. Antibodies against the antigen from the salivary glands or from whole larvae could not be detected by the used method in the sera of pigeons sensitized by adult feeding.

DISCUSSION

Our experiments demonstrated that a repeated feeding of *Argas polonicus* larvae on pigeons induces an immunoresistance of the hosts, which is manifested by a statistically significant decrease in the number of larvae capable of a successful termination of feeding. This adverse effect gradually increases up to the fifth infestation of pigeons when it remains constant at the level of about 90—95 % rejection of larvae. This gradual immunization suggests the chronic nature of argasid tick larvae bites. Johnston and Brown (1985) on the basis of reactivation of primary *Ornithodoros parkeri* feeding sites of nymphs and adults on guinea pig skin during the secondary feeding assume an apparent continual recruitment of basophils as a probable result of slow antigen release over time by appropriate sensitized antigen presenting cells, such as Langerhans cells or macrophages. The slow antigen release may also induce gradual increase in the host's immunoresistance after subsequent feedings of argasid larvae.

Shortening of the feeding period is another manifestation of the gradually increasing acquired resistance of the host. This phenomenon is certainly influenced by the fact that the slow-feeding larvae capable of terminating the feeding on naive or slightly resistant hosts are rejected by the resistant hosts on which only fast-feeding larvae are able to successfully finish their feeding. Nevertheless, the increased percentage of larvae detached on day 4 and 5 p.i. in resistant hosts suggests the existence of a mechanism accelerating the feeding of larvae on resistant hosts, i.e. the existence of a stimulating effect of the acquired host resistance on the speed of larval feeding. This is indicated also by the fact that the highest weight in the primary infestations was reached by engorged larvae of the Polish population detached on day 6 p.i., whereas from the fourth infestation the weight of these larvae was mostly lower than that of larvae detached on days 4 and 5 p.i. (Table 2).

The more expressive induction of acquired resistance of pigeons by the larvae of Polish population manifests a higher immunizing ability of these larvae, i.e. greater antigenic effect of their salivary gland products or ability to inoculate a higher amount of antigen into the host body during feeding. Similar intraspecific differences in the paralysis-inducing capacity of populations or strains have been reported in many species of ticks (Gothe and Bezuidenhout 1986, Gothe and Verhalen 1975). The latter authors, who studied the paralysis-inducing capacity of three *Argas* species, observed a distinct relation between the weight of the completely engorged larvae, probably determining the capacity of the oral toxin production, intensity of infestation, and clinical manifestation of the paralysis. In our experiments, the weight of larvae of the Polish population engorged on naive hosts was also significantly higher ($P < 0.01$) than that of larvae of the Czechoslovak population.

The slightly decreasing percentage of successfully moulted larvae with increasing number of infestations on the same pigeon was not statistically significant due to the small number of engorged larvae obtained from later infestations. It is probable that the highest mortality of larvae from later infestations was caused by the insufficient blood meal rather than the direct influence of the host's immune response. As it was demonstrated by Amin and Sonenshine (1970) in *Dermacentor variabilis* larvae, the blood meal insufficiency adversely affects the further development of these larvae causing a 3 times higher mortality than in the control group.

Repeated feeding of males and females did not induce any statistically significant adverse effect on the biology of ticks. The amount of blood engorged by the ticks was almost even during the whole experiment and the number of eggs deposited was directly proportional to the weight of engorged females. Not even the differences in the duration of preoviposition and preeclosion period or in the percentage of egg hatching were statictically significant. Similar results were obtained by Brown et al. (1983) and McLaren et al. (1983) who demonstrated that guinea pigs sensitized and challenged by the nymphs and adults of *Ornithodoros tartakovskyi* did not exhibit acquired resistance, even though their feeding induced a large blood and cutaneous basophilia. Brown et al. (1983) explicate this phenomenon by the fact that the cutaneous accumulation of basophils begins after the ticks have completed feeding or that the importance of the basophils and eosinophils is probably not reflected in their abundance in the blood.

The ingestion of sensitized basophils is probably not harmful to adult and nymphal argasids. On the other hand, Brossard et al. (1981) confirmed a little adverse effect on the biology of *Ornithodoros moubata* feeding on repeatedly challenged rabbit, such as smaller blood intake and longer feeding period. Although they demonstrated the presence of antibodies against salivary gland tissues in the rabbit serum by indirect immunofluorescence method, the relationship between the weight of engorged females and the number of eggs deposited remained linear, as in our experiment.

The detection of antibodies against antigens from whole larvae and salivary glands of adults in the sera of pigeons sensitized by larval feeding demonstrated that the acquired resistance of pigeons to *Argas polonicus* larvae is probably based on the immune response of the host. It demonstrated also the presence of antigen in the saliva of adult ticks. The fact that these antibodies were not detected in the sera of pigeons sensitized by feeding of adults might be caused by the use of an inadequate method. However, it is necessary to consider also the opinion of Brown (1985), who concludes on the basis of the studies by Trager (1940) and McLaren et al. (1983) that feeding of nymphs and adults of argasid ticks does not induce the acquired immune resistance, since these stages feed for a very short time (minutes to hours). Brown et al. (1983) assume that the strong cutaneous basophil response observed in some cases at the argasid feeding sites is not sufficient for the expression of their immune response. This is probably due to the fact that the cutaneous accumulation of basophils begins after the ticks have completed feeding. McLaren et al. (1983) observed at the feeding site of nymphal and adult *Ornithodoros tartakovskyi* mostly cytotoxic or degenerative alterations of basophils, which they suppose to be related with the lack of acquired resistance to nymphal and adult argasids. They assume that the anaphylactic type of basophil degranulation recorded at the rejection sites of hard ticks almost certainly represents a basophil against the tick effect, while the cytotoxic basophil alterations that accompany the lack of resistance to soft ticks may perhaps be indicative of a tick against basophil effect.

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ПРИОБРЕТЕННАЯ УСТОЙЧИВОСТЬ ГОЛУБЕЙ К ЛИЧИНКАМ *ARGAS POLONICUS*

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Резюме. Приобретенная устойчивость голубей к *Argas polonicus* проявляется неблагоприятным влиянием на питание и дальнейшее развитие личинок. После пяти последующих питаний отказ личинок достигал 90—95 % и средний вес накормленных личинок понизился с 2,18 мг на 1,72 мг у польской популяции клещей. У личинок из последующих инфестаций проявилась также тенденция к более быстрому питанию. Понизился также процент линявших личинок, вероятно вследствие недостатка корма на повторно сенсибилизированных хозяевах. Хозяева, сенсибилизированные личинками польской популяции клеща, проявили высшую устойчивость, чем хозяева, сенсибилизированные личинками чехословацкой популяции. Повторная сенсибилизация голубей не вызывала неблагоприятного действия на самцы и самки клещей. С помощью встречного иммуноэлектрофореза обнаружили антитела против клещей в сыворотке голубей, сенсибилизированных личинками, используя в качестве антигена экстракты из целых личинок или из слюнных желез взрослых клещей. В сыворотке голубей, сенсибилизированных взрослыми клещами, антитела не встречались.

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