RELATIONSHIPS BETWEEN THE DEFENSIVE SYSTEMS OF IBERIAN-BREED SWINE AND THE EUROPEAN VECTOR OF AFRICAN SWINE FEVER, *ORNITHODOROS ERRATICUS*

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ABSTRACT: To discover whether the immune system of Iberian-breed pigs exerts any adverse action on *Ornithodoros erraticus*, 3 pairs of pigs were subjected to a weekly infestation over 12 wk with 1,000 larvae, 500 nymphs-1, or 200 adults. Each pair was bitten by only 1 developmental stage. Batches of parasites identical to the foregoing ones were fed weekly on control swine. In none of the 10 parameters studied for each of the batches fed weekly was any significant difference found that could be attributed to the state of sensitization of the animals in which, in a previous study, the presence of high titers of anti-*O. erraticus* antibodies was found. It was observed that the possible pruritus due to immediate hypersensitivity reactions, which in the test animals appeared after the third week, had no protective value in the natural milieu. In view of the inability of the swine to exert any control over the soft ticks, it is concluded that the size of their populations in the pig pens and their composition according to the developmental stage are factors that depend exclusively on the opportunities that swine breeders offer such populations to feed on the animals.

A well documented fact in infestations by Ixodidae is that the protective value of the immune response developed by the hosts varies as a function of the parasite-host system in question (Ribeiro, 1987; Kaufman, 1989). In infestations by Argasidae, like the ixodid ticks, the development of a humoral (Brossard et al., 1981; Centurion et al., 1981) and cellular (Brown et al., 1983; Johnston and Brown, 1985) response has been reported in the hosts. However, in Argasidae in none of the host-parasite systems studied until now has it been possible to demonstrate that such responses have any direct protective action (Hoogstraal, 1985). The hosts used in work carried out with soft ticks were laboratory animals (rabbits or guinea pigs) that are artificial hosts for the species used in the tests. Accordingly, and in agreement with Willadsen and Kemp (1988), the results obtained in these hosts might not be truly indicative of what happens in natural host-parasite relationships. In this sense, Hoogstraal's (1985) observation that different results may be found in natural systems is suggestive. According to him, the most intense parasitism by *Argas (Persicargas) arboreus* affects the nestlings more than the adults of the heron *Bubulcus ibis ibis*, and this might be due to the existence in the adults of an immunologically mediated resistance.

In our field studies on the reservoir and European vector of African swine fever (ASF), *Or-*

nithodoros erraticus, we observed that the size of the populations infesting the pig pens and also their composition according to the ticks' developmental stages were factors that varied considerably from 1 swine-rearing farm to another. One cause of such variability could be the way in which pigs are handled on each farm; this could affect the opportunities of tick populations for feeding on swine that are their only hosts in Spain (Oleaga-Pérez et al., 1990). Another cause could be that the swine, after previous exposure to the parasites, might exert some regulatory action on such parasite populations.

The aim of the present work was to check experimentally whether in natural swine-soft tick systems the swine exert any immunologically mediated action on the life cycle of the parasites. To do so we started with knowledge of the existence of anti-*O. erraticus* antibodies in the swine used for feeding our soft ticks (Canals et al., 1990). One use of such antibodies is that they allow serological detection of the animals bitten by the parasite, thus making it possible to determine which farms harbor them. According to the results offered here, the immune response of swine against *O. erraticus* only has the aforementioned diagnostic value but is of no protective use because it is unable to affect the parasite negatively.

MATERIALS AND METHODS

Developmental stages and maintenance conditions

Developmental stages of *O. erraticus* employed for the sensitization of pigs were the larvae, nymphs-1, and adults of both sexes. All were from a colony es-

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established by us from specimens collected during the same year that tests were run on pig farms free of ASF in the province of Salamanca, Spain.

Larvae and nymphs-1 used in each infestation were of homogeneous age. This varied from 1 infestation to another, although attempts were made always to use specimens whose eclosion or molt had been verified during the 7 days prior to each test. Half of the adults used in each infestation were specimens captured in the field, and hence their physiological status was unknown; the other half comprised virgin specimens obtained at our laboratory from nymphs-4 and -5.

After separating the specimens by developmental stages both before and after feeding on the swine, they were kept in a climatized chamber (28°C, 85–90% RH, 16 hr light–8 hr darkness) in plastic vessels with a mesh top, with several discs of filter paper inside. Each vessel was labeled according to the batch of specimens that it contained, the pig on which the parasites had fed and the week during which they had done so, counted after the first week during which the pigs had been bitten. With no additional feeding, the parasites remained under these conditions until they either died or until 1 yr had elapsed since their feeding; at this moment the observations carried out on them were considered to have finished.

Hosts

Iberian-breed pigs free of parasites were used. Their weight range at the start of the test was 13–14 kg and about 40 kg at the end. Pigs 1 and 2 always were bitten by larvae, pigs 3 and 4 by nymphs, and pigs 5 and 6 by adults. Batches of parasites in the same 3 developmental stages were fed only once and at the same time on the control pigs that had the same characteristics as above and that were renewed weekly.

Procedure, number, and infesting doses

After immobilizing the swine on their backs, an open cylinder was placed on the ventral surface of the animals to afford access of the parasites to the swine. After 5 min the cylinder was removed and the specimens that had not attached to the animals' skins were removed. The feeding area was changed from 1 wk to the next and repeated every 3–4 wk. In the case of the infestations with adult specimens, the virgins were kept separate from those captured in the field.

Swine were infested once weekly over 12 consecutive weeks. The specimens fed on each pig were as follows: pigs 1 and 2, 1,000 larvae; 3 and 4, 500 nymphs; 5 and 6, 200 adults (100 from the field and 100 virgins; the same number of males and females). Specimens fed on each control pig were 1,000 larvae, 500 nymphs, or 200 adults.

Parameters of the parasites studied

The determinations carried out on batches of specimens fed over the 12 wk of the test were as follows: the time taken by the first and the 100th engorged soft ticks to abandon the host and the percentage of those abandoning their hosts over 1 hr with respect to the number of those placed on each pig; weight at 24 hr postinfestation (p.a.) of the first 100 ticks to abandon their hosts; larvae and nymphs-1 (the 100 first specimens fed) molted at 8 and 16 days p.a.; mortality of

nymphs-1 and -2 derived from the previous developmental stage from the first until the 12th mo p.a.; number of females that had begun oviposition at 12 and 19–21 days p.a. and the number of eclosed ovipositions at 26–27 and 33–34 days p.a. (in these determinations and the ones detailed below only the group of virgin females was employed to avoid the influence on the results of physiological heterogeneity of specimens captured in the field; in turn, in each batch of virgin adults the above-mentioned parameters were studied only in 5 subbatches, each of these comprising 1 female and 2 males); the number of eggs laid by the females from the previous experiments and the number of hatched eggs. The determination of the latter was carried out after sufficient time (≥ 3 mo) for all fertile eggs to have eclosed.

Parameters studied in hosts

In the pigs, apart from studying kinetics of the humoral (Canals et al., 1990) response, the existence or absence of erythematous reactions in the zones affected by the bites was recorded. Here we only report on such skin reactions because they may be indicative of immediate hypersensitivity reactions that have well known protective value against ectoparasites (Brown, 1988).

Statistical treatment

For each parameter studied a 2-way analysis of variance (ANOVA) was performed to observe whether there were any significant differences between the batches of ticks that had fed on the control swine and the sensitized swine and to see whether there were any significant differences among the different weeks of infestation.

In the event of any of such values having a $P < 0.050$, a 1-way ANOVA was carried out to discover the swine among which the significant differences had appeared and the week during which significant differences had occurred. Evaluation of the effect of time (week of infestation) on the parameters was done with the Scheffé F -test with a level of significance of 95%.

RESULTS

Larvae (Table I)

Significant differences were observed only in the time taken by the parasites to abandon their hosts in the first 100 fed specimens and in the percentages of fed specimens that had abandoned their hosts in the first hour. In the first case ($P < 0.05$) batches fed on the control swine fell off 10–13 min before those fed on pigs 1 and 2.

As a function of the week of infestation, the differences were significant only during week 9; in this week the times increased noticeably ($P < 0.05$) in all the swine, including the naive controls, such that this increase cannot be attributed to the sensitization of the animals.

Although the difference did not reach statistical significance ($P > 0.05$), the time taken by the first fed specimen to abandon its host also was shorter in the naive swine than in pigs 1 and

TABLE I. Values of parameters corresponding to batches of larvae fed once a week on naive swine or on pigs 1 and 2 sensitized for 12 wk with 1,000 specimens each time. Values are the means $\pm$ SD of 12 wk of infestation.

Host	Feeding and detachment time (min)		Detached during 1 hr (%)	Weight of 100 engorged larvae (mg)	Molts to nymphs-1 (%)			Cumulative mortality of nymphs-1 (%)			
	First specimen	First hundred			8 days	16 days	3 mo	6 mo	9 mo	12 mo	
Control swine	9.2 $\pm$ 4.5	19.8 $\pm$ 15.4	58.5 $\pm$ 33.8	38 $\pm$ 11	90.4 $\pm$ 19.3	98.3 $\pm$ 2.8	5.9 $\pm$ 11	24.1 $\pm$ 24.5	90 $\pm$ 12.8	100 $\pm$ 0	
Pig 1	11.3 $\pm$ 5.9	28.2 $\pm$ 18.2	50.8 $\pm$ 31	39.8 $\pm$ 1.6	92.8 $\pm$ 13	99.5 $\pm$ 0.9	1 $\pm$ 0.08	4.8 $\pm$ 3.8	66 $\pm$ 22.8	100 $\pm$ 0	
Pig 2	12.8 $\pm$ 4.5	33.3 $\pm$ 14.9	32.5 $\pm$ 21	38.2 $\pm$ 4.5	89.8 $\pm$ 20.3	99.6 $\pm$ 1.2	2.4 $\pm$ 2.7	21.9 $\pm$ 20.2	86.4 $\pm$ 23	99.2 $\pm$ 1.2	

2. A smaller number of specimens that fed for 1 hr and then abandoned their hosts was recorded on pig 2 than in the other 2, although this number was not modified significantly as a function of the week of infestation.

Nymphs-1 (Table II)

The time taken for specimen number 100 to abandon its host was on average about 14–22 min longer ($P < 0.05$) on pig 3 than on the naive controls or pig 4. The first specimen also took longer to abandon this pig than the other 2. In no case was there any increase ($P > 0.05$) in the delay as a function of the sensitization of the animals.

No significant difference was observed in the other parameters.

Adults (Table III)

The time taken for the first replete specimen to abandon the host was approximately twice as long ($P < 0.05$) in the case of pig 5 as compared with pig 6 and the naive controls, no difference being observed as a function of the week of infestation. A significant decrease was observed in the number of eggs per oviposition and the number of ecloded eggs ($P < 0.05$) with respect to other weeks in the batches of week 9, including the controls. Accordingly, the phenomenon cannot be attributed to sensitization of the animals.

A final observation, not shown in Table III, is that the number of specimens abandoning their hosts in 1 hr was on average 22% lower in the virgin adults than in adult specimens collected in the field.

Skin reactions in the pigs

In pigs 1–6, as from the third week postinfestation, a striking finding was the appearance of an erythematous reaction (Figs. 1–3) occurring at almost the same time that ticks attached to their hosts. The surface area of this reaction was larger than that occupied by the parasites and the reaction began to lose its intensity as the parasites fed, such that at the end of feeding only the actual bite sites were visible. In the naive swine no erythematous mark other than those corresponding to the bite sites themselves was observed (Fig. 4).

DISCUSSION

As reported by Canals et al. (1990), in pigs 1–6 the first anti-*O. erraticus* antibodies appeared

TABLE II. Values of parameters corresponding to batches of nymphs-1 fed once a week on naive swine or on pigs 3 and 4 sensitized for 12 wk with 500 specimens each time. Values are the means $\pm$ SD of 12 wk of infestation.

Host	Feeding and detachment time (min)		Detached during 1 hr (%)	Weight of 100 engorged nymphs-1 (mg)	Molt to nymphs-2 (%)		Cumulative mortality of nymphs-2 (%)			
	First specimen	First hundred			8 days	16 days	3 mo	6 mo	9 mo	12 mo
Control swine	6.5 ± 0.8	18.8 ± 15.7	55.4 ± 32	70 ± 8.7	52.1 ± 32.5	94.7 ± 13.7	1.7 ± 2.8	2.4 ± 2.7	12.2 ± 10	42.6 ± 25
Pig 3	12.1 ± 7	32.8 ± 18.2	64.6 ± 22	86 ± 9	44.7 ± 25.8	95.1 ± 10	2.8 ± 5.7	3.7 ± 6.7	9.8 ± 7.5	42.2 ± 15.1
Pig 4	6.5 ± 2.3	11.6 ± 4.3	77.1 ± 20	74.8 ± 10	50.2 ± 30.9	97.6 ± 7	3 ± 5.9	6.9 ± 12	20.7 ± 33	49.5 ± 27

TABLE III. Values of parameters corresponding to batches of adults fed once a week on naive swine or on pigs 5 and 6 sensitized for 12 wk with 200 specimens each time. Values are the means $\pm$ SD of 12 wk of infestation.

Host	Feeding and detachment time of first specimen (min)	Detached during 1 hr (%)	Ingested blood by specimen (mg)		% Females that had begun oviposition			Hatched ovipositions (%)		Eggs by oviposition	Unfertilized eggs (%)
			Females	Males	12 days	19-21 days	26 days	33 days			
Control swine	12.1 $\pm$ 11.8	58 $\pm$ 31	16.4 $\pm$ 2.7	3.4 $\pm$ 0.8	70 $\pm$ 21.6	98 $\pm$ 6.3	64 $\pm$ 20	98 $\pm$ 6.3	100.7 $\pm$ 33	5.32 $\pm$ 5.3	
Pig 5	24.9 $\pm$ 16.6	52 $\pm$ 32	18 $\pm$ 2.2	3.8 $\pm$ 0.9	56 $\pm$ 29.5	92 $\pm$ 10.3	66 $\pm$ 32	90 $\pm$ 14.1	114 $\pm$ 38	5.23 $\pm$ 11	
Pig 6	11.2 $\pm$ 5.7	71.5 $\pm$ 29	17 $\pm$ 2.7	3.7 $\pm$ 0.9	68 $\pm$ 21.5	94 $\pm$ 13.5	59 $\pm$ 29	90 $\pm$ 14.1	115 $\pm$ 43	7.62 $\pm$ 10	

during the second week postinfestation, their levels gradually increasing until maximum values of 6–8-fold the background value between week 7 and 13 of the test were reached. However, our results clearly indicate that the humoral response and foreseeably the cellular response accompanying it, has no deleterious action on the parameters studied, in agreement with what has been reported in artificial host–soft tick systems (Brosard et al., 1981; Johnston and Brown, 1985; Chinzai and Minoura, 1988).

Regardless of whether our batches of soft ticks had fed on sensitized or control swine, values for all the parameters studied showed great variability that could be attributed to 1 or all of the following 3 causes. The first cause could have been factors dependent on the pigs themselves. This is suggested strongly by the longer feeding times of the batches on pigs 3 and 5 from the first infestation onwards. Another cause could have been factors depending on the batches of parasites. In support of this is the work of Brosard et al. (1981) where feeding *Ornithodoros moubata* on genetically identical rabbits produced results as variable, or even more so, than ours. It is to this kind of factor that we attribute the significantly different results obtained in week 9. In view of the influence on some of the results from the virgin state of some adults, 1 of these factors might have been a small variation in the age/physiological state of the batches from week 9. Another factor might have been their genetic constitution. Although all the batches were from specimens captured in 1 place, the intraspecific variation of *O. erraticus* is very pronounced, even at the local level (Chabaud, 1954). Finally, we believe that the third cause of variation in our results was due to differences in temperature and light intensity (direct sunlight) of the premises where the infestations were conducted. It is probably not due to chance that values for parameters measured during the actual infestation procedure were subject to the greatest variation.

Although in no case can it be stated that variations observed would have been due to the state of sensitization of the pigs ($P > 0.05$), it cannot be ruled out that the state of sensitization might be the cause of some effect on the times of feeding and the percentages of specimens that fed more than 1 hr because it was for these, in general, that the greatest differences were observed between the sensitized and control swine. In the *Ornithodoros parkeri*–guinea pig system, Johnston and Brown (1985) also suggested that the

immune response would have been responsible for a significant effect on feeding time. However, we are inclined toward the notion that such an effect could be more beneficial than pernicious for the species, at least in the case of *O. erraticus*, because it would facilitate propagation of the species by pigs themselves among neighboring pens. In this sense, the behavior of some specimens in remaining attached to the swine even though they were completely full is probably an adaptation for the propagation of the species. In our tests, although some specimens abandoned their hosts as soon as they had fed, others that appeared to be full at the same time, as indicated by the first parasite abandoning its host (Tables I–III), remained attached to the swine for up to 1 hr longer.

Because there was no significant difference between the weight of the specimens that had detached from their hosts spontaneously and those taken off by us at the postattachment time, it seems clear that these second specimens do not remain on the swine to feed. It also seems clear that the behavior of these second specimens in no way affects their longevity, which was the same as that of the former specimens and in turn 3–4 times longer than that reported by El Shoura (1987) for specimens from Egypt kept under conditions identical to ours. Our interpretation is that the specimens that rapidly abandon their hosts are those destined to ensure the continuity of the species at the site where the parasite–host contact occurs, whereas those that persist on their hosts are destined to colonize other sites frequented by the swine.

In view of the little or no direct action of the defensive system of the swine on the soft ticks, it can be conjectured that size and composition by developmental stages of *O. erraticus* populations in pig pens depend exclusively on the availability of swine on which to feed. This availability now can be proposed as the last resource with which the defensive system of the swine might affect the parasites negatively.

As is well known, 1 of the results of immediate hypersensitivity reactions caused by the bites of hematophagous arthropods is the release of histamine into tissues, triggering the pruritus that typically accompanies such bites (Alexander, 1986). By inducing defensive behavior, the pruritus would be an important regulatory mechanism of the size of the parasite populations (Lloyd and Soulsby, 1988).

In swine sensitized, the immediate hypersen-

FIGURES 1-4. Skin reactions produced by *Ornithodoros erraticus* in pigs. 1. Erythema produced by 1,000 larvae that are not visible because blood ingestion had not begun. 2, 3. The same reaction illustrated in Figure 1 but produced by nymphs-1 and adults, respectively. 4. Larvae, nymphs-1, and adults feeding on a control pig. Note that in these erythema is circumscribed by the wheal corresponding to the bite.

sensitivity reactions probably appeared after the third week postinfestation, if the erythema that appeared when the specimens attached themselves to their hosts is considered indicative. If such an interpretation is correct, then under natural conditions the rapid appearance of pruritis could induce pigs to flee from pens where they were challenged by ticks.

In practice, however, we consider that the relevance of the above-mentioned protective mechanisms is null, or at least very small, because such a protective mechanism would be abolished when pigs are enclosed in pens and because in our field observations hundreds of ticks were seen biting swine spontaneously sheltering in their pens to which they had free access (or exit). It is possible that owing to their permanence in the infested zone such swine might have been in stage V of nonresponse, according to the reaction sequence described by Mellanby (1946) and Larivee et al. (1964). However, the advantage derived by swine from the establishment of such an unresponsive stage, because the bites can lead to their death (Oleaga-Pérez et al., 1990), is yet to be determined.

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