

ANNUAL REPORT TO THE U.S. AGENCY FOR INTERNATIONAL DEVELOPMENT
PROGRAM IN SCIENCE AND TECHNOLOGY COOPERATION

Project: Control of ticks and tick-transmitted diseases
by vaccination of the host

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This project had two major objectives: 1. To initiate studies toward the production of an anti-tick vaccine for use in ruminants (Research component), and, 2. To train a Dominican scientist in the corresponding bio-technology (Training component).

We are happy to report that we have made very satisfactory progress in both areas.

WORK ACCOMPLISHED.

1. RESEARCH COMPONENT.

1.1. Establishment of a valid laboratory model.

Our project was impaired from the beginning by the virtual absence of systematic knowledge about immunity to ticks in ruminants. Increasing evidence indicated that the immunological phenomena detected in traditional laboratory animals did not represent faithfully the immune responses of ruminants against ticks. Our first goal, then, was to develop a valid model of anti-tick immune resistance in ruminants that could be used in the laboratory, and a regimen of infestations that produced protective immunity consistently.

It took us two attempts but finally we found a model that produces consistent and increasing resistance at an excellent level for experimental work. This model is the infestation of a two year old Suffolk female sheep with 50 pairs of ticks (50 males and 50 females) at 0, 22, 78, and 105 days.

*Were other ticks fed on Tick-naive
Control ewes to give control for tick
parameters?*

1.2. Determination of the tick parameters affected by host's immunity.

Ticks have a rather complex life cycle constituted by a number of different phases that can be potentially affected by host's immunity. Since our model was of our own creation, there was no prior information about which phase was affected by host's immunity, or how it was affected. Since this information is fundamental for verification of immunity and for its later monitoring, we had to produce it by ourselves. Because the lack of previous work with this model prevented us from predicting which phases of the tick life cycle would be affected by immunity, we had to study all reasonable possibilities. We selected the following tick parameters: time of detachment, ticks existing at detachment, weight of the ticks at detachment, number of ticks that reach oviposition stage, time to beginning

of oviposition, weight of egg mass, weight of ticks after oviposition, time to hatch, and percentage of eggs that hatched. These values were recorded for each tick, analyzed statistically for the ticks of each infestation, and compared with those for the ticks of other infestations. The results in our preferred model appear in Table 1.

Table 1. Parameters of ticks during four successive infestations on a two-year old Suffolk female sheep.

<u>Tick Parameters</u>	1st inf	2nd inf	<u>P*</u>	3rd inf	<u>P*</u>	4th inf	<u>P*</u>
Engorged fem. wt.	615	578	.092	409	<.001	264	<.001
Fem. wt. aft. ovip.	252	188	<.001	178	<.001	153	<.001
Egg wt.	316	341	.214	207	<.001	91	<.001
Detachment day	12.9	21.0	<.001	20.7	<.001	26.3	<.001
Oviposition day	8.6	12.7	<.001	23.0	<.001	15.5	<.001
Percentage egg hatched	87	86	.445	79	<.001	59	<.001
% mortality to detachment	62	64	.385	73	.048	86	<.001
% mortality detach to ovip	3	6	.262	7	.183	43	<.001

If this ewe was her own control, seasonal variations could influence tick parameters. Was there a high-maintenance for control?

(*) As compared with the first infestation.

From this table, it can be seen that all the parameters studied are affected by host's immunity sooner or later. The weight of females after oviposition, the time to complete engorgement, and the time to oviposition are significantly affected already by the second infestation. The weight of the engorged females, the weight of the egg mass, the percentage of eggs hatched, and the mortality of ticks from application to detachment are significantly affected by the third infestation. The mortality of ticks from detachment to oviposition is reduced only by the fourth infestation.

It is also clear that time of detachment and time of oviposition are the easiest and earliest parameters to determine to verify immunity.

1.3. Determination of potential salivary antigens.

It is known that tick salivary glands enlarge and acquire secretion granules during the first three days of feeding. Since these secretions may have an important participation in the induction of host's immunity, our next task was to investigate whether this histological change had a functional expression in the potentially antigenic components of tick salivary materials. For this purpose, we collected salivary glands from unfed ticks and from ticks that had been fed for three days. Extracts of the respective glands were subjected to polyacrylamide gel electrophoresis in gradient gels, stained with silver, and compared for protein components.

We found that the unfed salivary gland demonstrated to possess 45 protein species whereas the fed one had only 42. Seven of the protein bands in the unfed gland were not present in the fed one, and five protein bands in the fed salivary gland were not present in the unfed one. These findings demonstrate that there is protein transformation during the early feeding of the tick but we still do not know whether this transformation is relevant to the production of protective immunity in the host.

1.4. Determination of active antigens in normal or immune sheep.

Our basic postulate was that the evidence of protective anti-tick immunity (as determined by the parameters explained under 2, above) should coincide with the appearance of antibodies to the antigens responsible for the protection. In order to identify these antigens, we performed Western blots of extracts of fed or unfed salivary glands with the serum obtained from the sheep before the first infestation, after the second infestation, and after the fourth infestation.

We found that the pre-infestation serum reacted with 27 antigens in the fed gland and with 13 in the unfed one. The post-second infestation serum revealed 31 antigens in the fed gland and 18 in the unfed one. The post-fourth infestation serum showed 31 antigens in the fed gland and 17 in the unfed one. Comparison of the patterns of fed or unfed gland extracts with the different sera revealed subtle differences in the antigens reactive on each occasion the interpretation of which will demand further work. In any case, we could identify six antigens in the fed glands and three in the unfed glands that were reactive with the post-fourth infestation serum but not with the pre-infestation serum. In all probability, the protective antigen (or antigens) is one of these.

Summary of the current achievements in research.

To this moment, we have created a valid laboratory model of anti-tick immunity in ruminants, have defined the parameters indicative of immune resistance to ticks in our model, have identified the antigens in fed and unfed tick salivary glands, and have determined the antigens that react only in immunoprotected sheep.

Since none of this information was known previously, we feel quite satisfied with the achievements of the project until now. The Dominican trainee, Dr. Andujar, presented our results at the Midwest Conference of Parasitologists in June, 1986 (see annexed photograph), and stirred a tremendous interest among our colleagues. During July and August, 1986, I will present our results to colleagues at the International Congress of

Infectious and Parasitic Diseases in Munich, Germany, and at Universities in Heilderberg, Germany; Zaragoza, Spain; Alfort, France; and, Lille, France.

2. TRAINING COMPONENT

Dr. Francisco Andujar was selected by the Dominican authorities for training because he is a veterinarian, he has field experience and a vast knowledge of the tick problems in the Dominican Republic, and he has the responsibility of applied tick research at the major veterinary laboratory of the Dominican Secretary of Agriculture.

Because upon his arrival to the United States he did not have the mastery of English regarded as essential for adequate training, he spent the first months of the project becoming proficient in the language.

He gained admission to the Ohio State University Graduate School later, and initiated academic studies and laboratory research in the subject of the project.

His courses were selected to give him a solid understanding of tick biology, pathology and epidemiology, of the immunological phenomena that occur in higher mammals, and of the basic sciences that support the modern knowledge in parasitology and immunology.

His laboratory work was designed to fit the needs of the project and, very particularly, the perceived needs of research in ticks and in general animal health in the Dominican Republic. In the specific area of tick research, he has learned how to identify ticks, how to reproduce their complete life cycle in the laboratory, how to infests different host, and how to verify the production of immune resistance. In the area of general research, he has learned immunological techniques, protein analysis of biological extracts, antigen analysis of complex antigenic mixtures, and is initiating work on antigen separation and characterization.

The emphasis of all this work has been on the understanding of the underlying biological phenomena, and on the meaningful analysis and interpretation of the information collected. The overriding consideration is to provide him with the kind of understanding that will allow him to do reliable research on his own, and to direct a research group upon his return to the Dominican Republic.

In our judgement, Dr. Andujar's progress during his training period has been outstanding. We believe that this part of the project has been extremely successful so far.

FURTHER WORK TO BE ACCOMPLISHED

1. Verification of the previous findings.

Although our findings to this moment seem very solid (see Table 1), we would like to repeat the multiple infestations in another sheep to verify the consistence of our model. We already secured a half-sister of our original sheep for this purpose. Since we must feed ticks on a sheep to obtain the salivary extracts necessary to continue the project, it will take little extra work to repeat new infestations according to the schedule used in our model. The repetition of the experiment, however, will make our work much more solid. In recognition of the advantages of confirming

results in repeated experiments, the original project included the study of more than an individual sheep for the definition of the model.

2. Study of intestinal antigens.

We have learned recently that a preliminary work done on cattle several years ago suggested that antigens from tick digestive tract might induce protective immunity in the host. Despite its relevance to our goals, this finding has not been confirmed. Since we will have tick materials and sheep sera, we would like to assay proteins of tick digestive tract against normal or immune serum by Western blot. The results will indicate whether digestive tract is a better source of protective antigens than salivary glands which is very important to our ultimate goal.

3. Separation of protective antigens.

This consists of the purification of the antigens found to be reactive only in sheep immunoprotected against ticks. This is a part of the original project that was described in the corresponding text.

4. Vaccination of sheep with the separated antigens.

This is also a part of the original project that was already described in the original text.

5. Initiation of work for the identification of antigens of Boophilus sp. and vaccination of cattle in the Dominican Republic.

This is also a part of the original project.

PROBLEMS ENCOUNTERED

Scientifically, the project has proceeded as expected and has yielded very important results already. Logistically, however, we had a number of set-backs.

First, we received notice of the awarding of the grant too late for Dr. Andujar to be adequately trained in English in the Dominican Republic. For this reason, we missed the first four or five months until he was ready to start working on the project. Second, a cut back of about 40% in the funds originally requested prevented us from acquiring the supplies and personnel to run more than one experiment at the same time. Since a series of five infections in sheep takes over 10 months, we spent almost the entire first year working with a model that finally proved to be inadequate. Under these conditions, attainment of only about 40 fed ticks to obtain salivary gland extract takes almost three months so the work is slowed down considerably.

Third, partially due to this delay the co-investigator that was supposed to provide the ticks (Dr. Needham) acquired new compromises and left the research team. This change was eventually beneficial to the project since it forced me to move the tick operation to my quarters which gives me a much better control over this part of the project. A slow down of tick

production for some time before Dr. Needham's final resignation, and the establishment of new quarters for ticks rearing consumed other few months of low productivity.

REQUEST FOR AN EXTENSION IN TIME WITHIN THE ORIGINAL BUDGET

In spite of the logistics set-backs, the project has proceeded very well and we have made important advances toward our original aims. Since we passed already the learning curve necessary for a new project of this nature, we feel that we are now much closer to our ultimate goal.

Continuation of this work is extremely important now since one of the three persons working on anti-tick immunity in ruminants in the United States quit recently (Dr. Steven Brown, of the University of Illinois). This leaves us and Dr. Steven Wikel (of the University of North Dakota) as the only groups doing research on this subject in the country.

For these reasons, we request an extension for an extra year (June 1, 1987, to May 31, 1988) to complete the work originally planned. We have administered our budget in such a way that we should be able to work this extra year within the limits of the budget originally approved.