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PROTECTIVE IMMUNE RESPONSES OF RABBITS TO *ASCARIS SUUM* LARVAL ANTIGENS

Parasitic nematode larvae frequently release physiologically active substances during and after hatching and moulting processes. These substances include enzymes which are often antigenic. Soulsby (1958) stated that most antigenic activity of *Ascaris suum* was associated with moulting. Sarles (1932) observed that *Trichostrongylus* larvae placed in immune homologous serum developed precipitates around various orifices such as the mouth, excretory pore and annus. Similar findings were reported for *Ascaris* and *Trichinella* (Mauss, 1941).

Mills and Kent (1965) reported that the antigens found in incubation media were excreted and/or secreted by *Trichinalla spiralis* larvae and were involved in the formation of immune precipitates. They demonstrated that these materials, when injected into mice, confer a certain degree of immunity.

Thorson (1956) studied the effect of serum from a dog resistant to infection with *Ancylostoma caninum* and found that it inhibited proteinase activity in esophageal saline extracts of adult hookworms. He demonstrated (1956a) that injection of the extracts into dogs inhibited the growth and maturation of the worms in a challenge infection.

Rogers (1958) described the hatching mechanism of *Ascaris lumbricoides* and revealed the presence of chitinase and esterase in the hatching fluid (H.F.) released by the larvae during hatching. Hinck and Ivey (1976) detected proteinase activity in *Ascaris suum* hatching fluid and in the excretions and secretions (E.S.) produced by the second stage larvae after hatching. Finch (1977) also found proteolytic activity in E.S. and somatic extract of *Ascaris suum* third stage larvae. Hinck (1971) demonstrated that both antihatching fluid and infection antisera inhibited hatching fluid proteinase. The present study was conducted to determine if immunization with *Ascaris suum* larval antigens provided any protection from infection.

Mature, female *Ascaris suum* were collected at a local abattoir. The methods for collecting, processing and embryonating eggs and the techniques employed for in vitro hatching and processing of hatching fluid have been previously described (Hinck and Ivey, 1976).

Third stage larvae were collected by infecting approximately twelve week old rabbits *per os* with 1,000,000 infective eggs. Viability was determined by microscopically examining the eggs for the presence of motile, second stage larvae. The per cent of infective eggs was determined and appropriate dilutions were made using deionized water. Five days post-infection the rabbits were sacrificed and the lungs removed aseptically. The lungs were placed in a Virtis homogenizer along with 0.85 per cent NaCl and homogenized at high speed for approximately 20 seconds. Larvae were recovered by using a modified Baermann apparatus. After two hours, larvae were collected and washed with sterile deionized water to remove erythrocytes and hemoglobin. Washed larvae were centrifuged and either disrupted by sonic oscillation or incubated to produce excretions and secretions.

Larval somatic extract was prepared by subjecting larvae, suspended in 0.85 per cent NaCl, to sonic vibration at 80,000 cycles per second with a sonic oscillator (Kewaunee Scientific Equipment) for thirty minutes. The sonicate was incubated at 4 C for 2 hours, centrifuged for 1 hour at 15,000 X G at 4 C, dialyzed against four changes of deionized water, lyophillized and stored at 4C.

Excretions and secretions of larvae were collected by incubating larvae for 24 hours at 38 C in sterile Eagle's Medium containing 10 mcg/ml Garamycin (Schering), added to inhibit bacterial growth. After incubation the E.S. was processed as previously described for the somatic extract.

Two month old rabbits were injected subcutaneously with 3.0 mg of a particular larval antigen reconstituted in 0.85 per cent NaCl mixed with an equal volume of Freund's complete adjuvant. After three weeks, those rabbits which produced a positive Arthus reaction were given a challenge dose of 100,000 infective eggs. Five days later the rabbits were sacrificed and the larvae recovered by the modified Baermann technique. The larvae were quantitated by making serial dilutions followed by a microscopic count of all larvae in 0.02 ml. This count was multiplied by the dilution factor to give a total recovery count. Controls of nonimmunized rabbits were infected for comparison.

Immunization of rabbits with various larval antigen preparations resulted in lowered numbers of third stage larvae recovered as compared to nonimmunized controls (Table). Immunization with hatching fluid produced a 19 per cent decrease in larval recovery while immunization with the second stage E.S. resulted in a 17 per cent decrease. Enzyme studies with hatching fluid and second stage E.S. revealed similar levels of proteinase activity in the two preparations (Hinck, 1971). Measured proteinase activity in third stage E.S., however, was nearly half that of H.F. and second stage E.S. while other enzymes found in H.F. and/or second stage E.S. were not detected at all in third stage E.S. (Finch, 1977). It seems possible that enzymes produced during hatching and for some time afterward by the hatched second stage larvae might be important for the early intrahost migrations of the larvae. After reaching the lungs and moulting to the third stage it is likely that invasive enzymes would be of less value. An immune response to the enzymes produced during the early phases of the intrahost migration would conceivably have a more protective effect than a response directed to the third stage E.S. This idea is compatible with the data since only a 7 per cent decrease of larval recovery was noted in rabbits injected with third stage E.S.

Immunization with somatic extracts provided essentially the same degree of protection as seen in the Table. With both second and third stage extracts, the decrease in larval recovery was approximately 25 per cent. It is possible that the antigenic makeup of both preparations is sufficiently similar so that immunization with either would provide protection against the early phase of the larval migration. Since the extract contains somatic antigens as well as enzymes released during homogenization, an immune response could be directed against the enzymes and against the larva itself. It would seemingly offer a number of possible mechanisms for interfering with its growth, migration, moulting, etc.

Table. Protective immunization with hatching fluid, excretions and secretions and somatic extract antigens from *Ascaris suum* developmental stages.

Sample	Viable larvae recovered after challenge[a]	% Decrease in larval recovery
Second stage		
Nonimmune control	26,780 ± 825	———
Excretions and secretions	21,817 ± 834	17
Somatic extract	19,320 ± 995	26
Hatching fluid	21,245 ± 716	19
Third stage		
Nonimmune control	23,089 ± 931	———
Excretions and secretions	21,387 ± 360	7
Somatic extract	18,364 ± 516	24

[a]Values represent the mean of five separate trials (± standard deviation).

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STATUS OF THE SMALL-FOOTED BAT, *MYOTIS LEIBII LEIBII*, IN THE SOUTHERN OZARKS

Although three vespertilionid bats of the southern Ozarks (the gray bat, *Myotis grisescens;* the Indiana bat, *M. sodalis;* and the Ozark big-eared bat, *Plecotus townsendii ingens*) are listed by the U. S. Fish & Wildlife Service as endangered, the small-footed bat, *M. leibii leibii*, is actually the rarest and least known bat occurring in the region. While the range of this bat is extensive (Hall, 1981), it is often regarded as the rarest bat of the eastern United States (Robbins et al., 1977). Interestingly, western subspecies are at least locally abundant (Webb and Jones, 1952; Farney and Jones, 1980).

Studies of the distribution and/or biology of the small-footed bat are hampered by the confusing nomenclature associated with earlier studies. Many workers in the past, and Hall recently, have utilized the specific epithet *subulatus* for the small-footed bat. This is confusing because prior to 1928, the specific epithet *subulatus* was applied to Keen's bat, *M. keenii*. We utilize the name *M. leibii* for the small-footed bat in compliance with recent checklists by Jones et al. (1979).