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IMMUNIZATION AGAINST LEPTOSPIROSIS: CONTINUED VACCINE TRIALS IN HAMSTERS USING STRAINS ISOLATED FROM BARBADOS[1]

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Heated whole-cell suspensions of L. interrogans *serotype* copenhageni *strains isolated from the field in Barbados have proved to be protective immunogens against experimental leptospirosis.*

Introduction

In a previous report (*1*) we described the preparation and effectiveness of heat-killed whole-cell bacterins derived from known laboratory model strains of virulent and avirulent *Leptospira interrogans* serotypes *canicola* (Moulton) and *pomona* (HCE). In the current investigation,[3] heat-killed whole-cell bacterins derived from *Leptospira interrogans* serotype *copenhageni* isolants from Barbados have been evaluated effective in immunizing hamsters against homologous challenge strains. Data obtained from all studies conducted thus far reveal that the concentration of heat-killed bacterins necessary to provide protection against experimental leptospirosis in hamsters is 1 μg if a virulent strain is used as a bacterin source and 10 μg if an avirulent strain is employed.

Materials and Methods

Organisms used in this study were kindly supplied by Mrs. C. June Roach of the Veterinary Diagnostic Laboratory, St. Michael, Barbados. Serotype identification was carried out by Mrs. K. Sulzer of the Center for Disease Control, Atlanta, Georgia. *Leptospira interrogans* serotype *copenhageni* isolants 354, 355, 375, 706, and 716 were isolated from rat (*Rattus rattus*) kidneys. Serotype *autumnalis* isolants 621 and 693 were isolated from the kidneys of a mongoose (*Herpestis auropunctatus*) and a rat (*Rattus rattus*), respectively.

All serotypes were maintained in bovine albumin polysorbate 80 (BAP-80) semisolid medium (*2*) and were transferred at 30-day intervals. Liquid BAP-80 cultures were prepared by inoculation of fresh semisolid BAP-80 and transferred, after 5-7 days' growth, to the appropriate volume of liquid BAP-80 medium. The incubation temperature was 30°C.

The heat-killed bacterin was prepared as reported in our previous publication (*1*). In the vaccine trials, 40g weanling female hamsters were divided into five groups of five animals. Each group was inoculated intraperitoneally with either 100, 10, 1.0, or 0.1 μg/ml of the heat-killed whole-cell bacterin. The control group received 0.85 per cent saline. All animals received 1 ml of fluid.

At 14 days post-inoculation each immunized and control hamster was challenged intraperitoneally with approximately $1x10^6$ cells of the homologous serotype. Post-challenge observation continued for 21 days. The kidneys and liver of each animal dying during the observation period were cultured. All the surviving animals were

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then killed and liver and kidney cultures were made. Serial dilutions of tissue homogenate prepared in a base of BAP-80 medium were inoculated into complete semisolid BAP-80 medium. The cultures were incubated at 30°C and examined weekly for 10 weeks. The presence of leptospiral cells, as observed by dark-field microscopy, indicated a positive culture. Neomycin sulfate and cycloheximide were not added to the isolation media.

All cell counts were determined by dark-field microscopy using a Petroff-Hausser counting chamber.

Results and Discussion

The results of this study, utilizing leptospirae isolated in the field in Barbados, are in agreement with our previous work (*1*) using laboratory model strains of *Leptospira*.

In an attempt to standardize the animal assay system for serotype *copenhageni*, isolant 375 was selected as the challenge strain and titrated in hamsters to determine the LD_{50}. The results are presented in Table 1. The LD_{50} was found to be less than 10 cells/ml; no attempt was made to determine the exact number. With the exception of isolant 354, all isolants of serotype *copenhageni* titrated similarly. Isolant 354 was found to be not lethal for hamsters, but did consistently establish the renal "carrier" state when inoculated into test animals. Experiments presently in progress will determine the effectiveness of the heat-killed whole-cell bacterin against establishment of the carrier state by this strain.

The results of the immunization trials using serotype *copenhageni* field isolant 375 from Barbados are presented in Table 2. These results are in agreement with our previous report (*1*) of work using laboratory model serotypes *canicola* Hond Utrecht IV and *pomona* S-91. The results are not in agreement, however, with the data previously reported for serotypes *canicola* Moulton and *pomona* HCE. Specifically, the heat-killed whole-cell bacterin prepared from serotype *copenhageni* protected against death and renal infection at the 10 μg dose level. At the 1.0 μg dose level, however, only 87.5 per cent of the immunized hamsters were protected against death and renal infection. As previously reported (*1*), bacterins prepared from *canicola* Moulton and *pomona* HCE protected 100 per cent of the animals at the 1.0 μg dose level.

A possible explanation for this variation in the results obtained with the bacterin prepared from serotype *copenhageni* field isolant 375 may be a reduction and subsequent loss of virulence. A loss of virulence was found when stock cultures were inoculated into hamsters. Of 10 stock cultures

Table 1. Summary of virulence titration data for serotype *copenhageni* isolant 375.

Cells per ml[a]	No. dead / No. tested	No. positive kidney cultures / Total No. kidney cultures	No. positive liver cultures / Total No. liver cultures	Death time (days)
$1x10^6$	10/10	10/10	10/10	7.0
$1x10^5$	10/10	10/10	10/10	8.0
$1x10^4$	10/10	10/10	10/10	12.0
$1x10^3$	10/10	10/10	10/10	12.5
$1x10^2$	10/10	10/10	10/10	13.5
$1x10^1$	10/10	10/10	10/10	13.5

[a] One ml inoculated intraperitoneally each into 40g weanling hamsters.

Table 2. Summary of immunization trials with serotype *copenhageni*[a] heat-killed whole-cell bacterin.[b]

Dose (μg per animal)	No. dead / No. tested	No. positive kidney cultures[c] / Total No. kidney cultures	No. positive liver cultures[c] / Total No. liver cultures	% positive cultures
100.0	0/20	0/20	0/20	0.0
10.0	0/20	0/20	0/20	0.0
1.0	5/20	5/20	0/20	12.5
0.1	20/20	20/20	20/20	100.0
Control group	20/20	20/20	20/20	100.0

[a]Mean death time for inoculation with $1x10^6$ cells/ml per animal of serotype *copenhageni* isolant 375 is 7 days.

[b]14 days after immunization, test animals and controls were challenged intraperitoneally with $1x10^6$ cells/ml per animal of serotype *copenhageni* isolant 375.

[c]After 21 days the post-challenge survivors were killed and cultures were made of kidneys and liver.

Table 3. Preliminary virulence titration data for serotype *autumnalis* isolant 621.

Cells per ml[a]	No. dead / No injected	Average death time (days)
$2x10^6$	4/4	3.5
$1x10^4$	2/2	7.0
$1x10^3$	4/4	7.5
$1x10^2$	4/4	8.0
$1x10^1$	4/4	8.5
$1x10^0$	4/4	9.0

[a]One ml inoculated intraperitoneally each into 40g weanling hamsters.

tested, 7 were found to be avirulent. It is possible, therefore, that the precise culture from which the bacterin was prepared was avirulent.

These data, however, still support our contention that heat-killed whole-cell bacterins prepared from field isolates provide protection against infection by the homologous serotype. Challenge of vaccinated animals with isolants 354 and 355 produced results consistent with those obtained using isolant 375; i.e., a 10 μg dosage was necessary to protect against death or renal infection. This is consistent with previous findings (*1*) that 10 μg of heat-killed bacterins derived from avirulent strains protected against challenge by virulent homologous serotypes.

Work is continuing on another field isolant from Barbados: *Leptospira* serotype *autumnalis*, isolant 621. Table 3 provides data on virulence titration of this strain which show it to be quite lethal. Vaccine trials are currently being conducted with this strain.

SUMMARY

Heated whole-cell suspensions of three field isolants of *L. interrogans* serotype *copenhageni* have been found effective in protecting hamsters against experimental leptospirosis. These data confirm the authors' previous work, in which heated whole-cell suspensions were shown to be protective immunogens. The ease and economy of preparing these immunogens suggest possible advantages to using such procedures in the field.

REFERENCES

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