IMMUNIZATION AGAINST LEPTOSPIROSIS: VACCINE TRIALS WITH HEAT-KILLED WHOLE CELL AND OUTER ENVELOPE ANTIGENS IN HAMSTERS[1]

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Two leptospiral antigen preparations were evaluated as vaccines. Heated whole cell suspensions proved very effective in protecting hamsters against experimental leptospirosis.

Introduction

Leptospirosis is considered to be the most important zoonosis in Barbados. Of the 88 clinical cases reported in 1970 and 1971, approximately 30 per cent were terminal (*1*). Subsequent serologic studies revealed that a very high percentage of the cattle surveyed had significant serum agglutination levels for *Leptospira* of the Autumnalis serogroup (*2*). Cultures of kidneys removed from trapped wild rodents yielded *Leptospira* strains of the serogroups Icterohaemorrhagiae, Ballum, Canicola, and Autumnalis—this last including a strain serotyped as *fort-bragg* (*1, 2, 3*).

The investigation reported below[3] was initiated in response to the leptospirosis problem in Barbados. It was necessary to first develop an effective vaccine prototype using known model strains prior to evaluating freshly isolated strains from Barbados.

Materials and Methods

Outer envelope preparations of *Leptospira interrogans* serotypes *canicola* Moulton, *canicola* Hond Utrecht IV, *pomona* S-91, and *pomona* HCE were obtained by the method of Zeigler and Van Eseltine (*4*). Briefly, the outer envelope was removed from the cell by osmotic shock, purified by isopycnic gradient centrifugation, and lyophilized. Serotypes *canicola* Moulton and *pomona* HCE are pathogenic for hamsters, whereas *canicola* Hond Utrecht IV and *pomona* S-91 are avirulent.

All serotypes were maintained in bovine albumin polysorbate 80 semisolid medium (*5*) and subcultured at 30-day intervals. Liquid cultures were prepared by inoculating fresh semisolid medium and then transferring to liquid medium after growth at 29°C for five to seven days.

Serial dilutions of the purified outer envelope preparations were made by resuspension of lyophilized preparations in sterile 0.85% saline. Heat-killed bacterins were prepared by sedimenting whole cells (log phase) from five-day cultures (cell count 10^9/ml) by centrifugation, resuspending the

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cell pellet in 50 ml of sterile 0.85% saline, and heating at 56°C for 30 minutes followed by lyophilization. For all the serotypes mentioned heat-killed bacterin and outer envelope preparations were made using in each case cells grown on the same batch of medium. Serial dilutions of the heat-killed whole cell bacterins were prepared in the same manner as the outer envelope materials.

In the vaccine trials the hamsters used were 40-gram weanling females separated into five groups of five animals each. The groups were inoculated intraperitoneally with 1,000, 100, 10, or 1 μg per animal of either the outer envelope or heat-killed whole cell preparation. Controls were inoculated with sterile 0.85% saline.

At 14 days after inoculation the immunized hamsters and the uninoculated controls were challenged intraperitoneally with approximately 2 X 10^6 cells/animal of the respective serotype. The challenged animals were observed for 21 days. The surviving animals were killed and their kidneys cultured. The kidney tissues were ground with a mortar and pestle in a mix of sterile sand in sterile phosphate buffer. Serial dilutions were inoculated into four tubes of bovine albumin polysorbate 80 semisolid medium. The cultures were incubated at 29°C and examined weekly for 10 weeks. The presence of a "Dinger's ring" in the culture tube and leptospiral cells, as observed by dark-field microscopy, was considered to indicate a positive culture.

Confirmation of leptospiral serotype was determined by the microagglutination test, as described by Galton et al. (*6*).

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

Results

The hamsters immunized with the outer envelope preparation derived from *canicola* Hond Utrecht IV were challenged with 2 X 10^6 cells/animal of virulent *canicola* Moulton (Table 1). Immunogen concentrations of 1,000, 100, and 10 μg/ml protected vaccinates against both death and renal infection. At the 1 μg/ml concentration, however, renal infection (but not death) was observed; 76 per cent of the kidney cultures were positive (19/25) at this concentration, whereas none were (0/25) at the higher levels. The heat-killed whole cell bacterin prepared from avirulent *canicola* was a more potent immunogen at the same dosage levels than the outer envelope preparations; only 20 per cent of the kidney cultures were positive (5/25) at the 1 μg/ml level.

Immunization with outer envelope and heat-killed whole cell preparations of virulent *canicola* Moulton was very effective, resulting in no deaths or positive kidney cultures at the end of the obvervation period.

The results from the experiments with virulent *pomona* HCE and avirulent *pomona* S-91 (Table 2) were similar to those obtained with the avirulent and virulent *canicola* strains. None of the animals immunized with virulent *pomona* HCE (either the outer envelope or heat-killed whole cell preparations) and challenged with the homologous organism gave positive cultures. On the other hand, those receiving *pomona* S-91 heat-killed whole cell bacterin produced 16 per cent positive kidney cultures (4/25) at the 1 μg/ml concentration, while those receiving *pomona* S-91 outer envelope produced 36 per cent positive cultures (9/25) at the same concentration.

Discussion

Generally, both the outer envelope and heat-killed whole cell preparations of avirulent and virulent leptospires proved to be potent immunogens. However, variations in potency were clearly revealed in the comparisons of bacterins or preparations made from the homologous virulent and avirulent strains of serotypes *pomona* and *canicola*.

Table 1. Summary of immunization trials with *Leptospira* serotype *canicola*.[a]

Immunizing agent	Dose (μg/animal)	Average no. positive kidney cultures[b]	positive kidney cultures
canicola Hond Utrecht IV (avirulent)			
Heat-killed whole cell preparation	1,000	0/25	0
	100	0/25	0
	10	0/25	0
	1	5/25	20
Outer envelope preparation	1,000	0/25	0
	100	0/25	0
	10	0/25	0
	1	19/25	76
canicola Moulton[c] (virulent)			
Heat-killed whole cell preparation	1,000	0/25	0
	100	0.25	0
	10	0/25	0
	1	0/25	0
Outer envelope preparation	1,000	0/25	0
	100	0/25	0
	10	0/25	0
	1	0/25	0
Control group	0	25/25	100

[a]At 14 days after inoculation the immunized hamsters and the diluent-inoculated controls were challenged intraperitoneally with 2×10^6 cells/animal of the virulent serotype.
[b]After 20 days the surviving animals were sacrificed and their kidneys cultured.
[c]Mean death time for *canicola* Moulton was 4.3 days for 2×10^6 cells IP.

Although all the preparations protected against death, renal infection was observed at the lowest dosage level with those derived from avirulent organisms. It is possible that loss of virulence may be accompanied by a corresponding reduction in antigenicity. At the lowest concentrations tested, the heat-killed whole cell bacterins appeared to be more effective in preventing renal infection than the outer envelope preparations. A possible explanation for the latter is that there may be varying amounts of different antigens in the heat-killed whole cell bacterins, which would not be the case with the relatively pure, probably type-specific antigen in the outer envelope preparations. Recent findings by others (*7,8*) have shown that the type-specific antigen resides in the outer envelope.

Bey et al. (*9*), using probit analysis, recently found a lower minimum effective protecting dose with an outer envelope bacterin tested in hamsters prepared by the method of Auran et al. (*10*) than that tested in the studies reported above (0.72 μg/animal as opposed to 1 μg/animal). Glosser et al. (*11*), using the outer envelope preparation as described by Auran et al. (*10*), found that a dosage level of 11.5 μg/animal protected against renal infection in hamsters. The antigens described by Bey et al. (*9*) were outer envelope and whole cell, the outer enveloped being chemically dissociated and reaggregated, while the whole cell preparation was made from lyophilized live cells. Their outer envelope antigen proved more effective than their whole-cell preparation.

Table 2. Summary of immunization trials with *Leptospira* serotype *pomona*.[a]

Immunizing agent	Dose (ug/animal)	Average no. positive kidney cultures[b]	positive kidney cultures
pomona S-91 (avirulent)			
Heat-killed whole cell preparation	1,000	0/25	0
	100	0/25	0
	10	0/25	0
	1	4/25	16
Outer envelope preparation	1,000	0/25	0
	100	0/25	0
	10	0/25	0
	1	9/25	36
pomona HCE[c] (virulent)			
Heat-killed whole cell preparation	1,000	0/25	0
	100	0/25	0
	10	0/25	0
	1	0/25	0
Outer envelope preparation	1,000	0/25	0
	100	0/25	0
	10	0/25	0
	1	0/25	0
Control group	0	25/25	100

[a]At 14 days after inoculation the immunized hamsters and the diluent-inoculated controls were challenged intraperitoneally with 2×10^6 cells/animal of the virulent serotype.
[b]After 20 days the surviving animals were sacrificed and their kidneys cultured.
[c]Mean death time for *pomona* HCE was 4.5 days for 2×10^6 IP.

On the contrary, in the experiments above the heat-killed whole cell bacterins proved at least as effective as the physically derived outer envelope preparations, or more so. By far, heating whole cells is the easiest, most economical method of preparing a bacterin; moreover, the results indicate that this would be the preparation of choice. These findings are consistent with those recently obtained by Ellinghausen and Painter (personal communication)—namely, that heated whole cell antigens are effective protecting immunogens.

The data presented herein are the results of immunization studies of whole cell and cell fraction bacterins prepared from serotypes known to be pathogenic for man and animals. The minimum dosage level required to protect against death and renal infection, and the duration of protection, are currently being investigated. Studies are also underway using heat-killed whole cell bacterins prepared from isolants received from Barbados.

SUMMARY

Heat-killed whole cell and outer envelope antigens prepared from homologous virulent and avirulent strains of *Leptospira* serotypes *canicola* and *pomona* were evaluated for protecting hamsters against experimental leptospirosis. The heat-killed bacterins proved at least as effective as the outer envelope antigens, or more so, in providing protection against death and infection, and they are easier and more economical to prepare.

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