Technical Note

Serologic response of llamas to a commercially prepared leptospirosis vaccine

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Abstract

Leptospirosis has been reported in llamas living in North America. Llamas in some locations are routinely vaccinated against leptospirosis in an attempt to prevent the disease. In order to determine if llamas respond to a leptospirosis vaccine, 14 llamas were immunized twice at 2 week intervals with a commercially available five-way leptospirosis vaccine. Blood was then collected for serum titer evaluation using a microagglutination technique for 5 months. Serologic response to the vaccine was low and of short duration to all five serovars, regardless of prevaccination titer. By 12 weeks postvaccination, only animals with serum titers > 1:200 to Leptospira pomona prevaccination still had serum titers > 1:200. Seven references are cited.

Keywords: Llamas; Leptospirosis; Vaccine

1. Introduction

Leptospirosis is rarely reported in llamas. Serologic evidence from South America indicates that the prevalence of exposure to leptospirosis in alpacas is about 3% (Thedford and Johnson, 1989). In the USA, leptospirosis caused the death of a guanaco (Hodgin et al., 1984) and an abortion outbreak was associated with rising titers to Leptospira grippotyphosa in a herd of 13 llamas. These abortions, which were associated with L. grippotyphosa, were apparently contained by vaccinating all breeding animals (Fowler, 1989). Commercially available multiple-serovar leptospira bacterins produced for other livestock species are used in llamas (Fowler, 1989). Vaccination is recommended where llamas are kept in endemic areas. However, little is known concerning the serologic response of llamas to leptospira vaccines (Long, 1989; Hill and Wyeth, 1991).

Serologic responses have been reported with bovine leptospirosis vaccines in alpacas (Hill and Wyeth, 1991) but only antitodal information exists from their use in llamas. Efficacy has not been assessed experimentally. The objective of this project was to determine the serologic response of llamas to a commercially available leptospira vaccine that contained antigens for L. grippotyphosa, Leptospira hardjo, Leptospira icterohaemorrhagica, and L. pomona.

2. Methods

2.1. Animals and vaccination schedule

Fourteen clinically normal, adult llamas were kept in three equalized paddocks (two paddocks each con-
taining five llamas, and one paddock containing four). The llamas ranged in age from 4 to 11 years and weighed 100–162 kg. Twelve of the llamas were intact males and two were geldings. Llamas were divided into two groups. The first group (five animals) was negative for antibodies (a titer of 1:200 or less) to the five serovars of *Leptospira* (*canicola*, *grippotyphosa*, *hardjo*, *icterohemorrhagica*, *pomona*). A serum titer of < 1:200 was set as the threshold for specific antibody response because of the background interference at low titer levels and because others (Carter, 1984) point out a 1:100 titer may be suspicious, but a > 1:100 titer is positive. This interference was possibly due to the factors such as non-specific reactions of llama serum antibody to antigens of the commensal leptospiras that are shared with the pathogenic leptospira serovars.

Llamas that had an antibody titer of ≥ 1:200 to any of the five serovars (*n* = 9) were placed in the second group. In this group, all nine llamas had titers to *L. hardjo* (1:200 to 1:1600) and five of the nine llamas had titers to *L. pomona* (1:400 to 1:1600). Llamas in this group were negative for antibodies to the other three serotypes of leptospirosis. None of these animals had been vaccinated for leptospirosis during the 2 years prior to this study. The vaccination history of these llamas 2 years prior to this test was unknown.

At the start of the study, 10 ml of blood were collected into a vacutainer. Concurrently, the animals were vaccinated intramuscularly in the rear leg with 2 ml of a commercially available multivalent leptospira vaccine approved for use in cattle and swine (5/2 ml; Norden Laboratories, Lincoln, NE).

This 2 ml dosage was the recommended cattle dosage. The vaccine was given by or under the close supervision of one of the authors (D.G.P.) and was given in the upper middle quadrant of the semimembranosus–semitendinosus muscle groups using 20 gauge 1 1/2 inch needles. Two weeks after the initial vaccination, blood samples were collected and the llamas were given a booster dose (2 ml intramuscularly) of vaccine. Blood samples were collected at 2 week intervals for 1 month after the second vaccination, followed at monthly intervals for 4 more months. Blood was allowed to clot, centrifuged at 2000 rpm for 15 min, serum harvested, and samples were frozen at −20°C prior to testing for antibodies for leptospirosis. Serum titers for leptospirosis were measured using the microagglutination technique. Screening of the sera and titration of the positive sera, along with running known positive and negative controls, followed the protocol described by Cole et al. (1973).

The llamas were observed daily for evidence of both systemic and injection site reactions to the vaccine, beginning the day of the first vaccination and continuing through 1 week after the second vaccination. The animals were observed for alterations in appetite, signs of depression, and injection site swelling and/or soreness.

### 2.2. Data analysis

For each week, the geometric mean titer (GMT) of each group of llamas was ascertained by determining the mean of the log₂ of the reciprocal of the titer. The GMT over time for each group of llamas (seronegative and seropositive) and each serovar were analyzed using the general linear model for repeated measures analysis of variance (SAS Institute Inc., 1989). All statistics were generated using SAS software (Version 6.03). If differences between the GMT for the seronegative and seropositive groups of llamas were not significant (*P* > 0.05), data were pooled for further analysis. A *P* value of <0.05 was considered to be significant.

### 3. Results

Results of the GMTs for each group are given in Table 1. Following vaccination, response to the five serovars was not significantly different (*P* > 0.05). Increases (*P* < 0.05) occurred in the GMT over time in antibodies to *L. canicola*, *L. icterohemorrhagica* and *L. pomona* (Table 1). Significant increases in GMT occurred most often in Week 4 postvaccination. Llamas never had an increase (*P* > 0.05) in GMT to *L. grippotyphosa* and there was a significant decrease in the GMT to *L. hardjo*.

The percentage of llamas from each group with titers ≥ 1:200 for each serovar by week are shown in Table 2. The highest percentage of llamas responding in both groups occurred in Week 4 postvaccination. Responses to *L. grippotyphosa* and *L. hardjo* appeared to be of shorter duration in both groups of llamas. By Week 16 postvaccination none of the llamas responded to either of these serovars with titers ≥ 1:200.
Table 1
Microagglutination titers in two groups of llamas (seropositive and seronegative) vaccinated on Weeks 0 and 2 with a five-way leptospirosis vaccine

<table>
<thead>
<tr>
<th>Week</th>
<th>Serotype</th>
<th>Canicola</th>
<th>Grippo</th>
<th>Hardjo</th>
<th>Ictero</th>
<th>Pomona</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Neg</td>
<td>Pos</td>
<td>Neg</td>
<td>Pos</td>
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<td>2</td>
<td></td>
<td>238</td>
<td>230 b</td>
<td>100</td>
<td>132</td>
<td>100</td>
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<tr>
<td>4</td>
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<td>303 b</td>
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<td>112</td>
<td>100</td>
<td>100</td>
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</table>

Data are given as the reciprocal of the geometric mean titer. n = 5 for the negative (Neg) group (n = 3 at W 16); n = 9 for the seropositive (Pos) group (n = 8 at W 16). Grippo, grippotyphosa; Ictero, Icterohemorrhagica.

a GMT of seropositive group greater (P < 0.05) than GMT of the seronegative group.

b Combined group GMT (seropositive and seronegative) different (P < 0.05) from GMT at Week 0.

Table 2
Percentage of llamas in each group (seropositive and seronegative) responding with a microagglutination titer ≥ 1:200 to each serovar by week

<table>
<thead>
<tr>
<th>Week</th>
<th>Serotype</th>
<th>Canicola</th>
<th>Grippo</th>
<th>Hardjo</th>
<th>Ictero</th>
<th>Pomona</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
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<td>Pos</td>
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<td>20</td>
<td>11</td>
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</tr>
</tbody>
</table>

n = 5 for the negative (Neg) group (n = 3 at W 16); n = 9 for the seropositive (Pos) group (n = 8 at W 16). Grippo, grippotyphosa; Ictero, Icterohemorrhagica.

Llamas that had titers ≥ 1:200 to one or more of the five serovars before vaccination (seropositive group) frequently had titers ≥ 1:200 to heterologous serovars at 2 and 4 weeks postvaccination. In this group of llamas, titers ranged from 1:100 to 1:1600 postvaccination. Five of the nine llamas in this group (55%) had titers ≤ 1:100 by Week 12 postvaccination and six of nine (67%) had titers ≤ 1:100 by Week 20 postvaccination.

Llamas that had titers ≤ 1:100 to all five serovars before vaccination (seronegative group) had a variable microagglutination titer response after vaccination. Titers in this group ranged from 1:100 to 1:1600 postvaccination; however, only one llama developed a titer of 1:1600 to L. hardjo 8 weeks after vaccination. Only two of five llamas in this group (40%) developed titers as high as 1:800 to any of the five serovars. By Week 12 postvaccination, three of five (60%) of these llamas had titers ≤ 1:100 to all five serovars. Four of five (80%) of the llamas in the seronegative group had titers ≤ 1:100 by 20 weeks postvaccination. One llama in the seronegative group never developed a measurable anti-
body titer to leptospirosis. No signs of swelling, pain, or other illnesses associated with the vaccinations were observed.

4. Discussion

There are several possible explanations for the poor antibody response of the llamas in this study, including use of a poorly immunogenic vaccine, use of an inappropriately low dosage of vaccine, or failure of the llama to respond to the vaccine given. No problems were noted with the serial number type of this vaccine (Dr. Ron Cravens, Smith, Kline, and Beecham, personal communication, 1993). The authors elected to give the llamas in this trial 2 ml of the vaccine, which is the recommended dosage for cattle that have a larger biomass than these llamas. The manufacturer also recommends this same dosage for swine, which is a divergent species and of different body size than cattle. No injection site reaction or other forms of adverse reaction were noted.

The immunologic response to *L. pomona* and *L. hardjo* to a commercially prepared leptospirosis vaccine given to alpacas was measured by New Zealand workers (Hill and Wyeth, 1991). Thirty-two per cent of their test alpacas showed no serologic response to *L. hardjo* up to 7 weeks after two vaccinations, which were spaced 2 weeks apart. Fourteen per cent of the animals developed initial serum titers of 1:100 or 1:200, which subsequently declined to 0. Only 47% of the alpacas in the New Zealand trial showed any serologic response to *L. pomona*, and from 4 to 6 weeks postvaccination the alpacas showing some serologic response declined to 37%. These workers noted a great deal of individual variation to vaccination (Hill and Wyeth, 1991).

Results from our study indicate the llamas in the seropositive group likely had previous exposure to *L. hardjo* and *L. pomona*. It is interesting to note that the highest prevaccination GMT occurred to *L. hardjo* (429); however, this did not appear to enhance the duration of the response to this serovar. By Week 12 postvaccination, all llamas in the seropositive group had titers ≤ 1:100 to *L. hardjo* and they remained negative to this serovar through Week 20.

The seronegative group of llamas seldom developed a GMT above the sensitivity threshold (≥ 1:200) of our testing methods. Significant increases in antibody titer (*P* < 0.05) did not occur beyond Week 4 postvaccination. By 12 weeks postvaccination only the GMT of seropositive llamas to *L. pomona* was ≥ 1:200, and by 20 weeks postvaccination the GMT of all groups was < 1:200. The serum titers to all serotypes were of short duration.

Although this trial did not evaluate efficacy through challenge with virulent leptospira, the authors still find it difficult to recommend this vaccine at this dosage for llamas until this vaccine has been shown to be of benefit through challenge studies. The poor serologic response in llamas indicates that llamas respond poorly to leptospira antigens in this vaccine. If this vaccine is used, it should be given frequently or strategically due to the short-term serologic response. Great individual variation may exist among llamas and alpacas with respect to response to leptospirosis immunization (Hill and Wyeth, 1991). Different strategies may need to be developed in areas where leptospirosis has been a clinical problem in llamas.

References


