Tick paralysis is caused by *Dermacentor andersoni* (Stiles) in the interior of British Columbia, Canada. Although this condition can be prevented by controlling *D. andersoni* with acaricides, the number of effective and registered compounds available in Canada has dwindled, providing impetus for development of alternative control methods. Recently, we demonstrated that cattle can develop immunity to tick paralysis after repeated exposure to paralyzing ticks. Gregson (1952) exposed 18 sheep to 1–10 female ticks on 38 occasions and produced six cases of mild paralysis (unsteady gait) and two cases of severe paralysis (hind limb ataxia). Several of these cases occurred in sheep that had been previously exposed to two to six ticks, leading Gregson (1952) to conclude that although individual sheep may be resistant to paralysis, it was not likely due to development of host immunity. However, 85% of the tick doses used by Gregson (1952) were less than the seven to 11 ticks per animal used by Hawden (1913) and Wilkinson (1985), which suggested that Gregson (1952) may not have used sufficient tick numbers to induce paralysis in his experiment, due to an earlier belief that single ticks could cause paralysis (Gregson 1973). Rigorous assessment of the development of host immunity requires infestation with a dose of ticks sufficient to cause paralysis in naïve animals. Although this dose has not been clearly established in sheep, Wilkinson (1985) suggested that dose–response studies in sheep should use ≈0.25–1.0 ticks per kg, whereas Gregson (1973) suggested the dose required for 50% paralysis (PD_{50}) was 30–40 female ticks.

The overall purpose of the present work was to determine whether sheep could develop immunity to tick paralysis caused by *D. andersoni*. Because earlier work on immunity used inconsistent doses of ticks, we first conducted a dose–response trial to establish a
relationship between the number of ticks and incidence of paralysis. A second experiment was conducted to determine if previously paralyzed sheep, and those passively immunized with serum from immunized cattle, were susceptible to a paralyzing dose of ticks.

Materials and Methods

Ticks. Ticks were obtained from a laboratory colony of paralyzing female *D. andersoni* maintained at the Lethbridge Research Centre (Lethbridge, AB, Canada). This colony had been selected over a number of generations to increase its ability to reliably produce paralysis (Lysyk and Majak 2003). Immature ticks were reared on rabbits, and adults were reared on cattle by using methods outlined previously (Lysyk and Majak 2003). Adult females were held at 10°C for at least 3 mo before use, to ensure attachment and increase the probability of causing paralysis. The use of animals for rearing ticks and for assessing tick paralysis, were reviewed and approved by the Lethbridge Research Centre Animal Care Committee.

Sheep. Arcott sheep were used for all experiments and were obtained from a flock maintained at the Lethbridge Research Centre. Sheep had been born and housed in a tick-free facility and had never been previously exposed to *D. andersoni*. Sheep were weighed and infested with ticks by using methods adapted from Wilkinson (1985). Wool was clipped from the backs of the sheep along the midline just behind the front shoulder, and the area washed with soap and water. Stockinet sleeves (QMD Medical, Montreal, QC, Canada) were fastened to the clipped area using contact cement (Helmetin, Toronto, ON, Canada). Ticks were placed inside the sleeves, which were knotted and opened only when ticks were to be removed. Sheep were housed in small metabolism crates (1 by 2 m) while infested. They had a collar that was chained to the front of the crate; the chain permitted some movement, but concurrently prevented them from chewing the sleeve. Infested sheep were observed twice daily for signs of paralysis (e.g., hind quarter ataxia and sternal recumbency). Ticks were manually removed upon first detection of paralysis or after 14 d in the absence of paralysis.

Experiment 1. Dose Response. The relationship between tick dose and the incidence of paralysis was determined in two separate trials conducted during February (winter trial) and June–July (summer trial) 2003. Twelve lambs used during the winter trial were each infested with either 2, 5, 10, 15, 20, or 25 unfed female *D. andersoni* and held until paralysis occurred or until 14 d had elapsed. Twenty-four lambs, ≈5 mo old and weighing 24.5 ± 2.3 kg, were used during the summer trial. Four sheep were each infested with either 2, 5, 10, 15, 20, or 25 unfed female *D. andersoni* and held until paralysis occurred or until 14 d had elapsed. The interval from infestation to paralysis was recorded, ticks removed, counted, and weighed individually.

The relationship between the incidence of paralysis and the number of ticks per sheep was determined using logistic regression (SAS Institute 2004), with the number of ticks on each sheep and body weight as separate covariates. A second logistic regression was conducted using the number of ticks/kg as the covariate. Sheep body weight was then tested to determine whether any residual effect remained after adjusting tick dose by body weight. The relationship between time to paralysis and tick dose was fit to the model $Y = \exp(a + b/(\text{ticks/kg}))$, where $Y$ is days to paralysis and $a$ and $b$ were parameters estimated using nonlinear regression (Systat Software, Inc. 2007). Factors influencing tick survival and individual tick weight were evaluated using backwards elimination stepwise logistic regression (SAS Institute 2004). Covariates evaluated were the number of days ticks had fed, the number of ticks per sheep, the number of ticks per kilogram, trial ($X_1 = 1$ for the winter trial and 0 for the summer trial), and the occurrence of paralysis ($X_2 = 1$ if paralysis occurred and 0 otherwise).

Experiment 2. Immunity to Paralysis. This experiment was conducted using 18 sheep, ≈5 mo old (body weight = 42.8 ± 3.3 kg). Six sheep, never exposed to ticks, were designated the naïve control group. Six sheep that were paralyzed during the summer trial were designated as the previously paralyzed group; they had been exposed to 20–25 ticks each and paralyzed in ≈8 d. A third group of naïve sheep received bovine serum collected from six cattle that had been exposed to paralyzing ticks on three occasions and had developed immunity to paralysis (Lysyk et al. 2009). Serum was collected in fall 2002 within 24 h after removal of ticks and stored at −20°C until August 2003. Serum was thawed, pooled, and ≈400 ml (1% of body weight) administered to each sheep over a 15-min interval, via an intravenous jugular catheter. During serum administration and for ≈2 h thereafter, sheep were carefully monitored for signs of adverse reactions.

To obtain serum for Western blotting, sheep were bled by jugular venipuncture immediately before tick infestation and 16 d after tick removal. Blood samples were held at room temperature for 1 h to allow clotting, and then they were centrifuged at 2500 × g for 15 min, and the serum was collected and stored at −20°C. All sheep were infested with a paralyzing dose of ticks (0.8 ticks per kg, based on experiment 1) and housed and handled as described in experiment 1. Ticks were removed when paralysis occurred or at 14 d after infestation, and then they were counted and weighed individually. The percentage of females alive when removed was determined, and the daily survival rate ($s$) calculated as $s = p^{t/4}$, where $p$ is the proportion alive and $t$ is days spent feeding. Fisher exact test was used to compare the incidence of paralysis among groups (Cytel Inc. 2007), and analysis of variance (ANOVA) was used to compare among groups for all other end points.

Western Blotting. Western blotting was used to determine whether sheep developed antibodies to specific tick salivary antigens and to compare this
response between paralyzed and nonparalyzed sheep. Paralyzing ticks were prefed on naïve cattle and assayed for paralysis on hamsters (Lysyk et al. 2005). Saliva was collected from paralyzing ticks after dopamine injection, as described by Kaufman (1978), and used as an antigen. Western blotting was conducted using methods outlined in Lysyk et al. (2009) for cattle serum, except that horseradish peroxidase-conjugated rabbit anti-sheep antibody (BioCan Scientific, Mississauga, ON, Canada) was used as the secondary antibody. Antibody reactions to antigens in electrophoretically separated paralyzing tick saliva were detected using an ECL Plus chemiluminescence kit (GE Healthcare, Little Chalfont, Buckinghamshire, United Kingdom). Molecular weights of the saliva antigens recognized were determined using Gel-Pro Analyzer 3.0 software (Media Cybernetics, Silver Spring, MD). The number of salivary antigens recognized by each individual sheep were compared among paralyzed and nonparalyzed sheep from all groups using two-way repeated measures ANOVA, with the occurrence of paralysis and sample (prechallenge and postchallenge) as main effects and sheep as subjects (PROC MIXED, SAS Institute, 2004). Measures of association between the recognition of a salivary antigen and immunity to paralysis were calculated for each band; these included percentage of agreement and Cohen’s kappa (Fleiss et al. 2003, Lysyk et al. 2009).

**Table 1. Relationship between the incidence of paralysis in sheep, tick dose, and sheep body weight**

<table>
<thead>
<tr>
<th>Dose variable ($X_i$)</th>
<th>$B_0$</th>
<th>$B_1$</th>
<th>$B_2$</th>
<th>$\chi^2$</th>
<th>df</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ticks</td>
<td>$-1.06 \pm 2.08$</td>
<td>$0.57 \pm 0.24$</td>
<td>$-0.23 \pm 0.10$</td>
<td>30.5</td>
<td>2</td>
<td>$&lt;0.0001$</td>
</tr>
<tr>
<td>Ticks per kg</td>
<td>$-6.03 \pm 2.51$</td>
<td>$14.29 \pm 5.46$</td>
<td>N.S.</td>
<td>29.5</td>
<td>1</td>
<td>$&lt;0.0001$</td>
</tr>
</tbody>
</table>

Dose was expressed as either the number of ticks per animal (Ticks) or the number of ticks per kilogram of sheep weight. The model is $P(\text{paralysis}) = 1/(1 + \exp(-(B_0 + B_1X_1 + B_2X_2)))$, where $P(\text{paralysis})$ is the incidence of paralysis, $X_1$ is dose variable, and $X_2$ is sheep weight (kilograms).

**Results**

**Experiment 1. Dose Response.** One sheep was eliminated from the winter trial due to an adverse reaction to handling. The remaining sheep had no adverse reactions, apart from paralysis, and all paralyzed animals recovered spontaneously within 24 h after tick removal. Logistic regression detected a significant relationship among the incidence of paralysis, the number of ticks per host, and sheep weight (Table 1). The effect of weight ($\chi^2 = 13.3, df = 1, P < 0.0003$) was reflected in the different responses between trials (Fig. 1A). However, adjusting tick dose by animal weight and expressing dose as ticks per kilogram removed differences between trials due to weight ($\chi^2 = 0.01, df = 1, P > 0.9$) and the incidence of paralysis increased with ticks/kg in a consistent manner across trials (Fig. 1B). The predicted dose for 50% paralysis was 0.42 ticks per kg. Furthermore, the probability of paralysis was predicted to exceed 98% at doses ≥0.7 ticks per kg. The effect of tick dose was also evident in the interval from infestation to paralysis; this interval declined from >12 d at 0.4 ticks per kg to <8 d at 1.3 ticks per kg (Fig. 2). The relationship between interval to paralysis and dose was days = $\exp(1.816 + 0.254/ (\text{ticks/kg}))$ [SE(a) = 0.073; SE(b) = 0.041; $r^2 = 0.74]$. Parameters were significantly different from 0 and 1, based on nonoverlap of 95% confidence intervals.

Based on stepwise logistic regression (backward elimination), tick survival was primarily influenced by

**Fig. 1.** Relationship between incidence of paralysis and the number of ticks per animal (A) and the number of ticks per kilogram (B). Solid squares, summer trial; open circles, winter trial. Lines are logistic regression (see text), solved at average sheep body weights.

**Fig. 2.** Relationship between interval from infestation to paralysis and the number of ticks per kg in sheep. Solid squares, summer trial; open circles, winter trial.
trial ($\chi^2 = 43.3, df = 1, P < 0.0001$) and not related to the number of days ticks fed, the number of ticks per sheep, the number of ticks/kg, or the occurrence of paralysis ($\chi^2 = 6.3, df = 4, P = 0.18$). Survival was greater during the summer trial (299/308 = 0.97) compared with the winter trial (133/170 = 0.78). The odds of surviving during the summer and winter trials were 33.2 (299/9) and 3.6 (133/37), and the estimated odds ratio was 0.11 (95% confidence intervals [CI] = 0.05–0.23).

Stepwise multiple regression (backward elimination) indicated that individual tick weight was not influenced by the number of ticks per sheep, ticks per kilogram, and the occurrence of paralysis ($F = 1.53; df = 3, 427; P = 0.20$). Tick weight was influenced by the number of days spent feeding and varied significantly between trials (Fig. 3). The model was milligrams of ticks = −98.9 (±13.1) − 51.4 (±9.1) × $X_1 + 25.8 (±1.4) ×$ days feeding ($F = 232.6; df = 2, 430; P < 0.0001; r^2 = 0.52$), where $X_1$ is 1 for the winter trial and 0 for the summer trial. The number of days spent feeding accounted for 48% of the variation in tick weight, whereas trial accounted for 14% of the variation in tick weight, but only added 4% to a model containing days feeding. The negative parameter for trial indicated that tick weights in the winter trial were ~52 mg less that in the summer trial, after adjusting for the number of days fed. Ticks gained ~26 mg/d.

**Experiment 2. Immunity to Paralysis.** There were no adverse reactions to the intravenous administration of bovine serum. The incidence of paralysis varied ($P = 0.002$; Fisher exact test) among the naïve, previously paralyzed, and serum-treated sheep. The incidence of paralysis was 100% in naïve sheep, which was greater than the incidence of paralysis in both the previously paralyzed ($P = 0.001$) and serum-treated sheep ($P = 0.03$; Fisher exact test). The average interval from infestation to paralysis was 8.3 d (range, 7–9 d) for naïve sheep, and 8 and 9 d for those treated with serum. Tick dose and animal weight were consistent among treatment groups (Table 2). The percentage of female ticks that survived, as well as the daily survival rate, did not vary among groups (Table 2) and the number of ticks per kilogram attached at the time of removed was also similar. The total weight of feeding female ticks, as well as average female weight, varied among treatment groups, and was lower in naïve sheep compared with those previously paralyzed or given serum.

**Western Blotting.** Western blots identified antibody responses to 14 tick salivary proteins. These had approximate molecular weights of 13.5, 17.9, 23.8, 28.1, 29.3, 30.6, 31.8, 33.9, 36.1, 38.1, 40.0, 43.3, 46.6, and 51.1 kDa. The number of antigens recognized in an individual sheep was variable, but increased between pre- and postchallenge samples ($F = 5.6; df = 1, 16; P = 0.03$). There was no significant variation in the number of antigens recognized by paralyzed and nonparalyzed animals ($F = 0.6; df = 1, 15; P = 0.45$), but there was a significant paralysis × sample interaction ($F = 4.3; df = 1, 16; P = 0.05$). This interaction indicated that variation in the number of antigens recognized in the pre- and postchallenge samples was not consistent among paralyzed and nonparalyzed animals. The number of antigens recognized by nonparalyzed (immune) sheep increased from an average ± SEM of 3.6 ± 0.6 in prechallenge samples to 5.5 ± 0.6 in postchallenge samples. The number of antigens recognized by sheep that were paralyzed (not immune) averaged 3.9 ± 0.6 and 4.0 ± 0.7 in the pre- and postchallenge samples, respectively.

Only a single band in the postchallenge samples was significantly associated with paralysis. Antibodies to the 43.3-kDa antigen had 72% agreement with immunity to paralysis (sensitivity and specificity of 0.60 and 0.88, respectively). Overall, the kappa value of 0.46 ±

![Fig. 3. Relationship between tick weight and the number of days ticks fed on sheep. Symbols denote averages. Solid squares, summer trial; open circles, winter trial.](https://academic.oup.com/jme/article-abstract/46/6/1436/923258/)

### Table 2. Host and infestation parameters for three groups of sheep exposed to paralyzing *D. andersoni*  

| Variable                  | Naive       | Previously paralyzed | Serum-treated | F      | P (>|F|) |
|---------------------------|-------------|----------------------|---------------|--------|---------|
| No. paralyzed             | 6           | 0                    | 2             |        |         |
| Sheep wt (kg)             | 42.6 ± 0.9  | 44.1 ± 2.0           | 41.9 ± 0.9    | 0.67   | 0.53    |
| Infestation dose (♀ per kg)| 0.50 ± 0.01 | 0.80 ± 0.01          | 0.50 ± 0.00   | 0.01   | 0.99    |
| % females alive           | 89.1 ± 3.9  | 89.6 ± 3.8           | 92.1 ± 1.9    | 0.23   | 0.50    |
| Daily survival            | 0.996 ± 0.005 | 0.992 ± 0.003    | 0.993 ± 0.002 | 1.02   | 0.39    |
| Live ticks per kg         | 0.71 ± 0.03 | 0.72 ± 0.03          | 0.74 ± 0.02   | 0.25   | 0.78    |
| Wt females removed (g)    | 3.7 ± 0.5a  | 8.0 ± 0.8b           | 6.7 ± 0.8b    | 11.28  | 0.001   |
| Female size (mg)          | 119.3 ± 8.0a | 251.3 ± 9.8e         | 212.2 ± 10.6b | 51.23  | <0.0001 |

Means ± SEM in same row followed by different letters are significantly different ($P < 0.05$). n = 6 except for female size (milligrams), where n = 188, 190, and 182 for naïve, previously paralyzed, and serum-treated groups, respectively. df = 2, 17 except for female size (milligrams), where df = 2, 557.
0.22 \,(Z = 2.05, P = 0.02)\) indicated a fair-moderate degree of association with immunity to paralysis.

**Discussion**

Differences between trials of experiment 1 in the incidence of paralysis most likely reflected differences in sheep body weights, because expressing tick dose in terms of ticks per kilogram removed any differences in dose response between trials. Future work should consider body weight of the host as a factor influencing paralysis. Wilkinson (1985) suggested that doses of 0.25, 0.5, and 1.0 ticks per kg could be used to develop a dose–response curve for tick paralysis and estimate the PD_{50}. We used a broader range of doses, especially at the lower end and found no paralysis at doses \(\leq 0.33\) ticks per kg. The incidence of paralysis increased rather steeply at larger doses, reaching 100% at \(\geq 0.8\) ticks per kg. Wilkinson (1985) paralyzed 50% of lambs (range in body weight, 23–34 kg) exposed to an average dose of 0.37 ticks per kg, close to our estimate of the PD_{50}. Hawden (1913) paralyzed sheep with seven to 11 lambs of I. {andersoni} \((as D. tennustus)\); however, the body weights of the sheep were not reported. Because his infestations were conducted during April and May, we inferred that he used spring-born lambs. The PD_{50} estimate of Gregson (1973) \((Z = 2.29, n = 10)\) after infestation (Doube and Kemp 1975), similar to the times reported here. Mice were killed after 4–5 d of feeding by I. {holocyclus} \((Goodrich and Murray 1978)\).

Variation in tick survival and weight between dose–response trials could have been due to the use of more mature sheep during the winter trial, but the contribution of environmental conditions cannot be discounted, because reduced temperatures can inhibit tick attachment and weight gain on large vertebrates \((Lysyk 2008)\). Although we have no record of indoor air temperatures during this experiment, outdoor maximum temperatures averaged 2.1°C during the winter trial and 26.5°C during the summer trial and undoubtedly resulted in cooler temperatures inside the barn during the winter. The facility is heated during the winter but not cooled during the summer. The effect on paralysis, however, was relatively small because adjusting for sheep body weight removed any significant variation between trials in dose response.

Previously paralyzed sheep were immune to paralysis at a dose that was capable of paralyzing those that were naive. Previously paralyzed sheep had been exposed to tick doses ranging from 0.81 to 1.30 ticks per kg during initial infestation and a nearly uniform dose of 0.8 ticks per kg at the second infestation. Sheep passively immunized with serum from immune cattle also developed partial immunity to paralysis. Immunity may have resulted from the introduction of antibodies that inhibited the paralysis toxin as it was introduced to the sheep, or by inhibiting production of toxin within the feeding ticks as host IgG ingested by feeding ticks can be transported into the salivary gland \((Wang and Nuttall 1999)\). Hyperimmune serum against I. {holocyclus} toxin does not provide cross-protection against paralysis caused by D. {andersoni} \((Gregson 1973)\), indicating these toxins are probably different toxins.

Variation in paralysis among groups cannot be attributed to variation in tick dose or sheep body weight, because these factors were consistent among treatment groups \((Z = 2.29, n = 10)\). Tick doses used were obviously sufficient to cause 100% paralysis in naïve animals. However, care must be taken to distinguish whether immunity developed to the paralysis toxin itself or to tick attachment and feeding \((Bagnoli and Doube 1975)\). Immunity to tick attachment and feeding can be expressed as reduced feeding success or female weight \((Allen and Humphreys 1979, Whelen et al. 1986)\). Tick survival was similar among groups, with no indication of immunity. Females in the two treated groups were larger than in the naïve group. The relationship between tick size and time spent feeding developed for the naïve animals predicted an average weight of 115.2, 262.3, and 223.6 mg for ticks removed from the naïve, previously paralyzed, and serum-treated animals, respectively. These values were similar to average tick weights for all groups \((Z = 2.29, n = 10)\), indicating no reduction in tick growth due to host immunity.

The assertion that sheep did not develop immunity to tick paralysis was probably based on experiments using inadequate initial doses of ticks \((Gregson 1952)\). In that study, severe paralysis occurred in one lamb that had two previous exposures to five and one ticks, respectively, and in another that had previously been exposed to two doses of a single tick each. Two cases of mild paralysis occurred in lambs that had been previously exposed to two or three females. Immunity may not have developed because the initial infestations might not have used virulent ticks, or because doses of tick insufficient to cause paralysis do not induce immunity. In the present experiment, a single manifestation of severe paralysis accompanied by hind limb ataxia was sufficient to impart immunity. Whether low doses that do not cause paralysis stimulate the development of immunity remains to be determined.

Sheep developed an antibody response to a variety of tick salivary antigens, similar to previous observations with cattle \((Lysyk et al. 2009)\). However, immune sheep developed antibody response to an additional two antigens during tick exposure, whereas cattle developed antibody response to an additional five antigens during tick exposure \((Lysyk et al. 2009)\). Four salivary antigens had excellent agreement with immunity to paralysis in cattle \((Lysyk et al. 2009)\), whereas only a single salivary antigen had a fair-to-moderate association with immunity to paralysis in sheep. This antigen had a molecular weight of 43.3 kDa, similar to the largest \((42.2 \text{ kDa})\) reported for...
cattle. The cause of this apparent difference among host species is unknown, but may include some experimental effects, because cattle were exposed to paralyzing ticks up to three times in previous work, whereas sheep were only exposed once or twice.

Passive immunization afforded only partial protection against paralysis. This method for protecting animals is not practical because producing paralyzing ticks is time-consuming. However, vaccination with inactivated toxin may be a valid approach for controlling tick paralysis in western North America. The feasibility of developing a vaccine would depend on the ability to identify and isolate the toxin. Efforts should focus on identifying salivary antigens of ~40–44 kDa.

In conclusion, the PD\text{50} value for *Dermacentor andersoni* in Arcott sheep was 0.42 ticks per kg body weight. Previously paralyzed sheep were not paralyzed after subsequent exposure, whereas passive immunization conferred protection against paralysis in only some sheep. Sheep paralyzed after tick exposure developed antibodies against *D. andersoni*; antibodies to a 43.3-kDa antigen had 72% agreement with immunity to paralysis, and a sensitivity and specificity of 0.60 and 0.88, respectively. Active immunization has the potential to protect animals against paralysis.

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