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NOTES

Correlation between DNA Restriction Fragment Length Polymorphisms in *Leptospira interrogans* Serovar Pomona Type Kennewicki and Host Animal Source

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Isolates (n = 147) of *Leptospira interrogans* serovar pomona type kennewicki from cattle, swine, horses, and wildlife were analyzed by DNA restriction endonuclease analysis. Restriction fragment length polymorphisms were identified in DNA digested with *Hpa*II, and the restriction fragment length polymorphisms were correlated with the host animal source of the isolates. These results will be useful in understanding the epidemiology of serovar pomona infections in livestock.

Leptospirosis, caused by infection with *Leptospira interrogans* (sensu lato), is a common zoonotic disease which affects most species of mammals. Infection of swine with *L. interrogans* serovar pomona may result in abortions, stillbirths, and neonatal deaths (4, 12). Infected pigs usually survive the acute stage of the disease and shed serovar pomona in their urine. Swine, skunks, and opossums often shed serovar pomona in their urine for months after infection and serve as natural reservoir or maintenance hosts for this organism (4). Serovar pomona is also commonly isolated from cattle (4–7, 12) and horses (2) and is occasionally isolated from humans (7), sheep, and various wildlife species (4). Infection of these incidental hosts often results in severe disease and usually occurs as a result of contact with infected pigs or other reservoir hosts or their urine (5, 6, 8, 12).

Thiermann et al. (13) examined 25 isolates of serovar pomona from North America and Australia by restriction endonuclease analysis (REA) of chromosomal DNA. The fragment patterns produced when DNAs from these isolates were digested with *Eco*RI, *Bgl*II, *Hin*dIII, or *Hha*I differed from the fragment pattern of the reference strain for serovar pomona, but the patterns were identical to those of the serovar kennewicki reference strain. Because serovar kennewicki is no longer recognized as a distinct serovar of *L. interrogans*, these organisms were identified as serovar pomona type kennewicki (13). No differences in the restriction fragment patterns obtained with these enzymes were detected among the 25 isolates of kennewicki. Skilbeck et al. (10) analyzed 20 serovar pomona isolates from Australia and New Zealand by REA with *Eco*RI and also identified the isolates as type kennewicki. Two variant profiles were detected in the isolates from Australia and New Zealand, indicating that there was some heterogeneity among the type kennewicki isolates (10). In the study described here, we analyzed by REA 147 isolates of serovar pomona type kennewicki from North and South America and found a correlation between digestion fragment profiles and the host animal source.

Isolates (n = 147) of serovar pomona type kennewicki were obtained from various hosts, from animals with severe disease and subclinically infected animals, and from 18 states in the United States, 3 provinces in Canada, and Chile (Table 1). Leptospires were grown in liquid BAP-80 medium (3) at 30°C, and chromosomal DNA was extracted as described previously (9, 13). Purified leptospiral DNA (2 μg) was reacted with 4 to 5 U of either *Eco*RI or *Hpa*II (Bethesda Research Laboratories, Gaithersburg, Md.) for 3 h as directed by the manufacturer. After the addition of tracking dye, the digestion products were separated by electrophoresis in 0.7% agarose (Marine Colloids, Rockland, Maine) with Tris-borate buffer for 16 h at 60 V. The gels were stained with ethidium bromide (0.25 μg/ml; 45 min) and photographed under short-wave UV light through a Kodak 23A red filter.

All isolates were identified as serovar pomona type kennewicki on the basis of *Eco*RI digestion profiles. The isolates restriction fragment length polymorphism patterns were identical to that of the kennewicki reference strain, but they differed from that of the pomona reference strain (Fig. 1). The *Eco*RI digestion profiles of the isolates examined in the present study were similar to those previously described by others (1, 10, 11, 13).

When leptospiral DNA was digested with *Hpa*II, six distinct profiles were seen and were designated profiles 1 to 6 (Fig. 1). This result indicates that serovar pomona type kennewicki is a genetically heterogeneous group of organisms. In several cases, we examined multiple isolates from the same herd or outbreak of pomona infection. In each instance, organisms isolated from the same herd had identical profiles when they were digested with *Hpa*II (data not shown). This suggests that the genetic heterogeneity is not caused by inherent genetic instability or

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TABLE 1. Host and source of isolates of *L. interrogans* serovar pomona

Host species	No. of herds	No. of isolates	Source[a]
Porcine	30	37	1, 3, 4, 5, 6, 10, 11, 12, 13
Bovine	52	57	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12
Equine	35	43	1, 4, 5, 14
Wildlife[b]	3	10	1, 14

[a] 1, National Animal Disease Center, Ames, Iowa; 2, J. G. Songer, Tucson, Ariz.; 3, C. Armstrong, West Lafayette, Ind.; 4, National Veterinary Services Laboratory, Ames, Iowa; 5, J. Prescott, Guelph, Ontario, Canada; 6, C. Kirkbride, Brookings, S.D.; 7, F. White, Gainsville, Fla.; 8, L. Hanson, Urbana, Ill.; 9, Centers for Disease Control and Prevention, Atlanta, Ga.; 10, B. Kingscote, Lethbridge, Alberta, Canada; 11, R. Higgins, St. Hyacinthe, Quebec, Canada; 12, S. Reidemann, Valdavia, Chile; 13, H. Hill, Ames, Iowa; 14, P. Donahue, Lexington, Ky.

[b] Four skunks, two feral cats, and four raccoons trapped in the immediate vicinity of infected horses (skunks and cats) or swine (raccoons).

selective pressure during an outbreak. For further analysis, all isolates from the same herd were considered to be one isolate.

The profiles of the type kennewicki isolates were then analyzed for a correlation between the profile and the host from which they were isolated (Table 2). The majority (77%) of the type kennewicki isolates from swine had *Hpa*II digestion profile 1, with a few isolates having profile 2. In contrast, only 13.5% of the isolates of type kennewicki from cattle had profile 1. Profiles 2 and 3 were the most common among isolates from cattle. The correlation of host and restriction fragment profiles was consistent in cattle and swine, despite the broad geographic distributions of the isolates.

Most of the equine isolates (91%) had REA profile 2. However, 40 of the 43 equine isolates were from horses in central Kentucky, and therefore, the geographic diversity of the isolates was small. Type kennewicki isolates from horses in Canada, Oklahoma, and California (one isolate from each location) had REA profile 1. Analysis of a larger number of isolates from horses will be necessary to document the incidence of the different REA profile types in horses.

The wildlife isolates of type kennewicki were rather evenly divided between REA profiles 1 and 2. Isolates were obtained

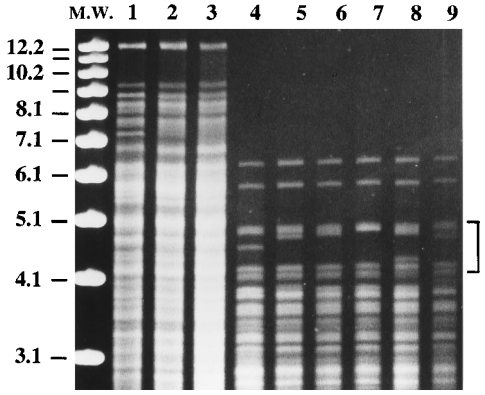

FIG. 1. REA profiles of DNAs from reference strains and isolates of *L. interrogans* serovar pomona type kennewicki. M.W., molecular mass ladder (in kilobases); lane 1, *Eco*RI-digested DNA from serovar pomona reference strain; lane 2, *Eco*RI-digested DNA from serovar pomona type kennewicki reference strain; lane 3, *Eco*RI-digested DNA from serovar pomona type kennewicki isolate; lanes 4 to 9, *Hpa*II-digested DNAs from serovar pomona type kennewicki isolates with profiles designated 1 through 6, respectively. The bracket indicates the areas of difference between the profiles.

TABLE 2. Restriction endonuclease *Hpa*II digestion profiles of serovar pomona type kennewicki isolates by host species

Host species	No. (percent) of animals with the following REA profile:					
	1	2	3	4	5	6
Porcine	23 (77)	7 (23)				
Bovine	7 (13.5)	28 (54)	9 (17)	6 (11.5)	1 (2)	1 (2)
Equine	3 (8.6)	32 (91.4)				
Wildlife	4 (40)	6 (60)				

from skunks ($n = 4$) and feral cats ($n = 2$) trapped on horse farms that were experiencing outbreaks of leptospirosis. The REA profiles of the type kennewicki isolates from the skunks and feral cats were identical to those obtained from the horses on these farms. Likewise, isolates of type kennewicki from raccoons ($n = 4$) that were trapped on swine farms or on the premises of swine abbatoirs had REA profiles that were common to infected swine at those locations. These findings further demonstrate the stability of the genetic profiles among isolates involved in a single outbreak.

The correlation between host and restriction enzyme digestion profiles of leptospiral DNA has not been reported previously. It is possible that several type kennewicki variants, with different but stable REA profiles, exist and circulate primarily in swine or cattle populations. However, we would expect more frequent intermixing of the variants in the environment because of the common contact between swine and cattle or their waste via shared pastures or water supplies. It is also possible that the restriction fragment length polymorphisms identified in the present study are the result of host adaptation of serovar pomona type kennewicki. There may be a direct link between the profiles and the genetic differences that make the variants more able to survive and grow in different hosts.

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