
SHORT REPORT

Serological prevalence to six leptospiral serovars in cattle in Asturias (Northern Spain)

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SUMMARY

The prevalence of antibody to six serovars of *Leptospira interrogans* in cattle in Asturias (Northern Spain) was determined by the microscopic agglutination test (MAT). Using 50% agglutination or lysis at a dilution of 1:10 or more as the criteria for seropositivity, 371 of 3578 (10.36%) animals were found to react with one serovar. The most commonly detected serovars were *pomona* (5.59%) and *grippityphosa* (2.37%), whilst serovar *hardjo* (0.75%), *icterohaemorrhagiae* (0.64%), *poi* (0.64%) and *autumnalis* (0.36%) were found at lower frequencies.

Infection of cattle with serovars of *Leptospira interrogans* is an important economic problem in many countries throughout the world and constitutes a public health risk among particular occupational groups. Few surveys of leptospiral infection have been carried out in Spain, either in humans or cattle. Human leptospirosis in Spain was traditionally considered a disease endemic almost exclusively in rice workers [1], but more recent surveys have shown that other activities involving contact with animals or marsh waters have a similar level of risk [2]. Serological surveys of Spanish cattle detected *pomona* and *icterohaemorrhagiae* antibodies in 7.9% and 7.1% of cattle slaughtered at Barcelona and Badalona abattoirs, respectively [3], while antibodies to 14 *Leptospira interrogans* serovars were detected in 46.5% of 560 sera of cattle slaughtered at Cádiz abattoir, with *icterohaemorrhagiae* and *pomona* the most prevalent serovars [4]. This study, however, concerns cattle in Asturias in Northern Spain, where milk production is more common and the environmental conditions might be more favourable to the survival of leptospires.

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The Asturian brucellosis eradication scheme requires annual blood sampling of all cows over 6 months of age. Between January and December 1993, 397823 blood samples were submitted to the Asturias Animal Health Laboratory in weekly batches. Sampling was done on a geographic basis, so each batch consisting of up to 20000 samples collected from animals from a particular locality. Sera were stored frozen at -20°C until used. Among these sera we applied unbiased systematic sampling to select 3578 samples representing nearly 1% of the cattle population of the Asturian region.

MAT were performed as described by Wolff [5] using the following reference strains of *L. interrogans*: *autumnalis* (Akiyami A.), *grippityphosa* (Moskva V.), *icterohaemorrhagiae* (RGA), *poi* (*poi*), *pomona* (*pomona*) and *hardjo* (*hardjoprajtino*), provided by H. Korver. Sera were initially diluted at 1:10 and screened by incubation with antigens for 2 h at 37°C . Sera-producing agglutination were subsequently retested using serial dilutions.

Data were stored and analysed by means of the epidemiological computer program EPI INFO [6]. Significance of changes in prevalence were tested by χ^2

for discontinuous and the Student's *t* and ANOVA for the continuous variables (age and herd size).

Table 1 summarizes the results of the MAT. Using 1:10 as the cut-off for seropositivity, antibody to one or more serovars was detected in 371 (10.4%) of the 3578 sera investigated, and with 1:80 as the cut-off 199 (5.6%) were positive. The most prevalent serovars detected were *pomona* (5.59% at 1:10) and *grippotyphosa* (2.37%), while serovar *hardjo* (0.75%), *icterohaemorrhagiae* (0.64%), *poi* (0.64%) and *autumnalis* (0.36%) were less common. The seroprevalence of each serovar was compared by means of $\chi^2_{(Yates)}$, and this showed significant differences between the prevalences of *pomona* and *grippotyphosa* serovars ($P < 0.001$), and between these two serovars and all the rest ($P < 0.001$). Figure 1 shows the geographic distribution of the six serovars in the Asturian area. Cattle reacting with serovar *pomona* were found throughout the region, while serovar *grippotyphosa* reactors were found mainly in the southwest. Serovar *hardjo*, although having a low overall prevalence (0.75%), had 10% prevalence in one area. A higher seroprevalence (14.6%) was found in the local beef breed, 'Asturiana de los Valles', than in Friesian dairy cattle (9.5%), although this was only significant ($P < 0.001$) for serovar *grippotyphosa*. The prevalence of antibody did not significantly vary with cattle population's age.

The 1:10 dilution was chosen as the cut-off for seropositivity because higher dilutions may not detect antibodies at a very early stage of infection [12] and may underestimate the true seroprevalence of some host-adapted serovars [13]. Moreover, positive reactions due to vaccination were not expected since leptospiral vaccines have not been used in Asturian cattle. Despite this, the seroprevalence of the six serovars studied (10.36% at 1:10, 5.6% for 1:80) was much lower than that found in other countries [7-11]. This difference was largely due to the low seroprevalence of serovar *hardjo* in Asturias (0.75%).

The geographic distributions of the animals in Asturias seropositive to different serovars may be related to differences in the distributions of maintenance hosts, which could not be dealt with in this study. The most common reported reservoir of serovar *grippotyphosa* in some European studies is the common vole (*Microtus arvalis*) [14, 15], whereas the wild boar may be the maintenance host in some areas of Spain [16].

Further work is required to determine whether leptospirosis is a frequent cause of bovine abortion in

Table 1. MAT titres of sera for six leptospiral serovars in cattle in Asturias

| Serovar | Number Examined | Number Positive | Percentage positive | Agglutination-lysis titres number positive (Titres \geq 1:10) | | | | | | | | | | |
|-----------------------|-----------------|-----------------|---------------------|---|------|------|------|-------|-------|-------|--------|--------|--------|---------|
| | | | | 1:10 | 1:20 | 1:40 | 1:80 | 1:160 | 1:320 | 1:640 | 1:1280 | 1:2560 | 1:5120 | 1:20480 |
| <i>Pomona</i> | 3578 | 200 | 5.59 | 9 | 33 | 47 | 42 | 36 | 14 | 9 | 2 | 5 | 1 | 2 |
| <i>Grippotyphosa</i> | 3578 | 85 | 2.37 | 2 | 20 | 20 | 19 | 8 | 7 | 3 | 6 | — | — | — |
| <i>Hardjo</i> | 3578 | 27 | 0.75 | 2 | 1 | 6 | 7 | 4 | 3 | 2 | 1 | — | — | 1 |
| <i>Icterohaemorr.</i> | 3578 | 23 | 0.64 | 1 | 7 | 3 | 4 | 2 | 2 | — | 3 | 1 | — | — |
| <i>Autumnalis</i> | 3578 | 23 | 0.64 | — | 3 | 5 | 4 | — | — | — | 1 | — | — | — |
| <i>Poi</i> | 3578 | 13 | 0.36 | — | 3 | 10 | 5 | 3 | 1 | — | 1 | — | — | — |
| Total | 3578 | 371 | 10.36 | 14 | 67 | 91 | 81 | 53 | 27 | 14 | 14 | 6 | 1 | 3 |

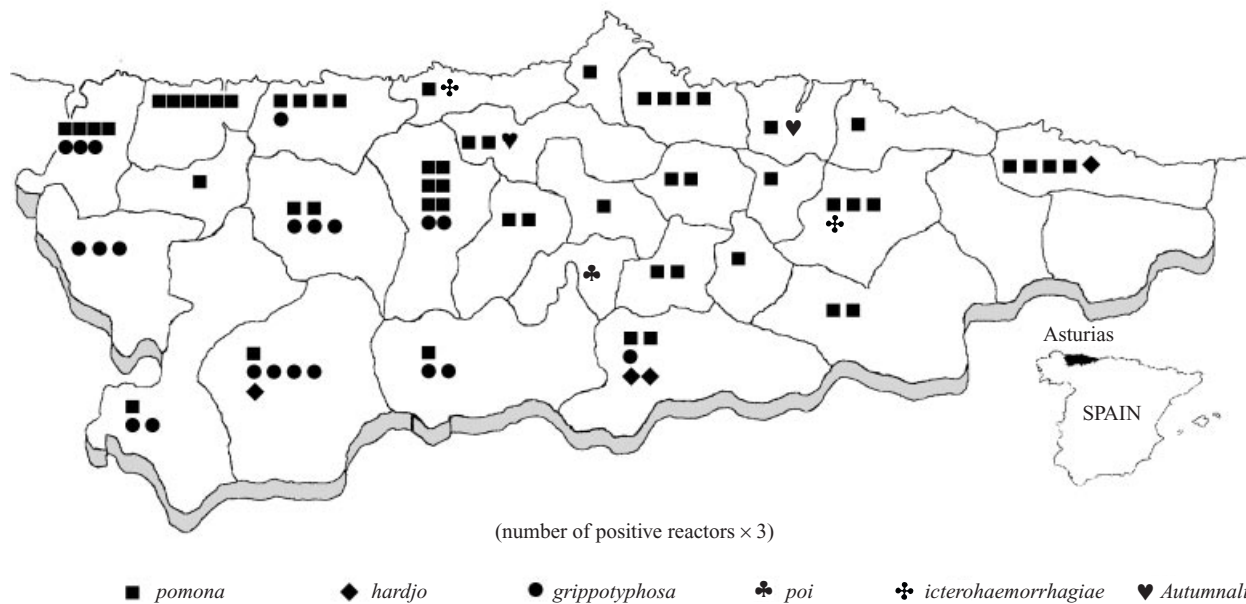


Fig. 1. Geographic distribution of seropositive animals against each one of the six investigated leptospiral serovars in cattle in Asturias (Titres \geq 1:10).

Asturias. From our data, Asturian cattle appear to be seldom exposed to leptospiral infection and therefore vaccination programmes could be restricted to infected herds or those with clinical disease. If only sporadic cases arise, it might be more profitable to attempt to dispose of reactors to remove carriers rather than rely on vaccination. The role of cattle and other species as sources of human leptospirosis in Asturias remains unknown and requires further study. However the data for acutely ill, hospitalized patients, suggest that infection is more commonly acquired from feral than from domestic animals (Consejería de Sanidad, unpublished data), which is consistent with the results of this survey in cattle.

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REFERENCES

1. Gimeno de Sande A, Pumarola A. La leptospirosis en los arrozales de España (Encuesta Epidemiológica Nacional). *Rev Sanidad Hig Públ* 1967; **41**: 1–15.
2. Dastis C, Villar E, Marín I, et al. Prospective serological study of leptospirosis in Southern Spain. *Eur J Epidemiol* 1996; **12**: 257–62.
3. Covaleda J, Pumarola A. Encuesta serológica en el ganado equino y bovino sacrificado en los Mataderos Municipales de Barcelona y Badalona. Existencia de aglutininas frente a especies de leptospiras diversas. *Rev Ibér Parasitol* 1956; **16**: 315–8.
4. León Vizcaino L, Miranda A. Investigación epidemiológica sobre leptospirosis en Cádiz. *Bol Consejo Gen Col Vet* 1976; **30**: 15–32.
5. Wolff JW. The laboratory diagnosis of leptospirosis. Illinois, USA: Thomas Springfield, 1954.
6. Dean AG, Dean JA, Burton AH, Dicker RC. EPIINFO, version 5: a word processing, database and statistics program for epidemiology on microcomputers. USD Incorporated. Stone Mountain, Georgia, EEUU, 1990.
7. Ellis WA, O'Brien JJ, Cassells J. Role of cattle in the maintenance of *Leptospira interrogans* serotype *hardjo* infection in Northern Ireland. *Vet Rec* 1981; **108**: 555–7.
8. Miller DA, Wilson MA, Beran GW. Survey to estimate prevalence of *Leptospira interrogans* infection in mature cattle in the United States. *Am J Vet Res* 1991; **52**: 1761–5.
9. Brieger C., Brack A. *Leptospira interrogans* serovar *hardjo* in cattle: prevalence and importance as an abortion agent in Switzerland. *Schweizer Archiv Tierheilkunde* 1986; **128**: 43–4.
10. Milner AR, Wilks CR, Calvert K. The prevalence of

- antibodies to members of *Leptospira interrogans* in cattle. Aust Vet J 1980; **56**: 327–30.
11. Collares-Pereira M. Bovine leptospirosis in cattle in Portugal: bacteriological and serological findings. Vet Rec 1991; **128**: 549–50.
 12. Faine S. Leptospira and leptospirosis. Boca Raton, FL, U.S.A: CRC Press, 1994.
 13. Prescott JF. Leptospirosis. In: Jubb KVF, Kennedy PC, Palmer N eds. Pathology of domestic animals, 4th ed. London: Academic Press Limited, 1993; **2**: 503–10.
 14. Borcic B, Kovacic H, Sebek Z, Aleraj B, Vrtkovic N. *Leptospira interrogans* serovar *grippotyphosa* in small wild mammals in northern Croatia. Veterinarski Archiv 1987; **57**: 319–29.
 15. Kuiken T, van Dijk JE, Terpstra WJ, Bokhout BA. The role of the common vole (*Microtus arvalis*) in the epidemiology of bovine infection with *Leptospira interrogans* serovar *hardjo*. Vet Microbiol 1991; **28**: 353–61.
 16. León Vizcaino L, Miranda García A, Perea Remujo A, et al. Investigación inmunoserológica de diversos agentes infecciosos en ciervos y jabalíes de Sierra Morena. In: Castroviejo, J ed. Proceedings of the 'II Reunión Iberoamericana de Conservación y Zoología de Vertebrados'. Cáceres, España, 1983: 490–502.
 17. Ellis WA. Leptospirosis – A review of veterinary aspects. Irish Vet News 1990; **12**: 6–12.