Serologic Diagnosis of Leptospirosis: Comparison of Line Blot and Immunofluorescence Techniques with the Genus-Specific Microscopic Agglutination Test

Colleagues—Leptospirosis is a widespread acute febrile zoonotic disease that is usually detected serologically by the microscopic agglutination test (MAT) [1]. In recent years, many new techniques using an enzyme-linked immunosorbent assay (ELISA) have been reported. These include Dot-ELISA [2, 3] and Dig-ELISA [4], which are effective but do not enable simultaneous detection of several antigens.

We assessed several techniques for their suitability for testing a battery of antigens in nonspecialized laboratories: a microimmunofluorescence (MIF) technique, an ELISA that was adapted from the dot-blot and called "line-blot" (D. Raoult, G. Dasch, unpublished data), and the MAT.

A total of 173 serum samples were obtained from the French National Reference Center for Leptospirosis. By clinicoepidemiologic data and by MAT 97 samples were negative, 3 were weakly positive (1:100 to 1:200), and 73 were positive or strongly positive (>1:400). Antibodies were primarily to Leptospira icterohaemorrhagiae (36), L. grippotyphosa (31), and L. australis (9). Four strains, L. icterohaemorrhagiae copenhageni, L. grippotyphosa, L. australis australis, and L. biflexa patoc, were used and purified by successive centrifugations in phosphate-buffered saline (PBS).

In the line-blot, we apply a line of antigen with a pen holder at 0.5-cm intervals on nitrocellulose paper. The paper is then cut perpendicularly in 0.5-cm-wide strips that are tested as in Western blotting. The antigen is first solubilized in 8% sodium dodecyl sulfate, 2% mercaptoethanol, 2 Tris NaCl (pH 6.8), and bromophenol blue (0.02%).

We tested the four antigens by line-blot and traced a line with PBS alone as a negative control. The sheet was blocked 1 h in Tris-buffered saline (TBS) and 5% nonfat dry milk, rinsed, and stored. Sera were used as in the dot-blot [3, 4] with a single 1:25 dilution but tested separately with antibody to human immunoglobulin (IgG or IgM). The line was read optically and considered positive when obviously darker (figure 1) than the negative control. As in MIF, the four antigens were dispensed with a pen, and a final titer in IgG and IgM was determined, beginning with a dilution of 1:25. A titer of 1:100 in IgG and 1:50 in IgM was considered positive.

For sensitivity we compared the MIF test and the line blot for (1) specific serovar, (2) the nonpathogenic strain L. biflexa patoc, and (3) the four pathogenic strains. By MIF, 59 serum samples had IgG antibodies to the specific serovar, 56 had antibodies to the nonpathogenic strain, and 63 had antibodies to one of the other strains. For IgM antibodies the respective results were 54, 51, and 55. When both IgG and IgM antibodies were considered, the numbers of positive samples were 68 (89%), 66 (87%) and 71 (93%), respectively.

By line-blot, IgM antibodies were found more frequently than were IgG antibodies in all three comparisons: for specific serovar, 60 versus 49; for the nonpathogenic strain, 59 versus 33; and for any of the four pathogenic strains, 64 versus 51. IgG or IgM antibodies were found in the respective tests in 71 (93%), 66 (87%), and 74 (97%) of the samples.

Of the 98 serum samples negative by MAT, by MIF 7 had either IgG or IgM antibodies, 7 had antibodies to L. biflexa patoc, and 11 had antibodies to one of the four pathogenic strains. By line-blot, the respective numbers of antibodies were 10, 3, and 13.

The dot ELISA using a single nonpathogenic strain [3] or both pathogenic and nonpathogenic strains [2] has been proposed as a screening test. We suggest that the line-blot be used to test several antigens. We selected the three common pathogenic serovars in France, but as many antigens as desired can be assessed with this method. We added L. biflexa patoc as the most commonly used strain for screening tests [1], and our results with the line-blot are good if we consider the sensitivity (97%).

Had we tested only for L. biflexa patoc strain, 66 (87%) of the sera would have been considered positive, which is an argument for multiple testing of antigens. Although the line-blot is not very specific and we have a 13% false-positive rate, we consider this technique a screening method. The line-blot technique is easy to perform, and the strip can be stored and provide a permanent record. The technique could be adapted to any geographic area for antigen selection, and no specific material or competence is required. The overall results with the MIF are comparable to those of the line-blot; however, it is not as easy to perform for inexperienced personnel and it requires a fluorescent microscope.

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Toxocarasis Possibly Caused by Ingesting Raw Chicken

Colleagues — The number of cases of toxocarasis caused by the larval migrations of *Toxocara canis* and *T. cati* within the viscera of humans is increasing worldwide [1, 2], including in Japan [3]. Humans become infected by ingesting infective eggs or by eating the meat of paratenic hosts containing encapsulated larvae. The latter is believed to be very rare, but in 1988 we encountered two cases of toxocaric infection possibly caused by ingestion of raw chicken giblets. The patients were 22-yr-old Japanese twin brothers, residing in Kanagawa Prefecture, Japan, with a history of good health. In a snack bar on 20 January 1988, they dined on fresh slices of raw liver and gizzard of a fighting cock. Soon after eating, both patients vomited. The next morning they complained of abdominal discomfort. One week later they developed urticaria with fever to 38°C.

Hematologic studies on the first admission (5 February 1988) revealed a leukocytosis of 13,200/mm³ (case 1) and 23,600/mm³ (case 2), with eosinophil counts of 60% and 84%, respectively. These counts decreased gradually, and 3 w later the number of leukocytes was within normal limits. The concentrations of serum gammaglobulins were remarkably increased, to 23.8% (case 1) and 35.2% (case 2). Both patients were hypergammaglobulinemic as follows: case 1, 2,350 mg/dl, 378 mg/dl, and 1,820 IU/ml, and case 2, 4,170 mg/dl, 239 mg/dl, and 6,450 IU/ml of IgG, IgM, and IgE, respectively. Clinical laboratory data were all within normal limits, but both patients had mild liver disturbances revealed by elevation of alanine and aspartate aminotransferases. Chest radiographs, stool examinations, and physical, neurologic, and ocular findings were unremarkable.

The clinical features mentioned above were consistent with the symptoms of *Toxocara* visceral larva migrans (VLM) described by Arean and Crandall [4]. In order to confirm the clinical diagnosis of *Toxocara* infection, enzyme-linked immunosorbent assay, indirect fluorescent antibody test, counter immunoelectrophoresis, and immunoblotting were performed. The results of these tests confirmed infection with *Toxocara* but failed to distinguish between *Toxocara* species. The specific diagnosis of *T. canis* toxocarasis was based on Ouchterlony’s diffusion-in-gel tests using a battery of helminthic antigens. After the specific diagnosis of VLM, oral mebendazole was prescribed (300 mg three times daily for 4 d). Although the number of leukocytes remained within normal limits, the eosinophilia and hypergammaglobulinemia persisted. On 3 October 1988, 9 mo later, eosinophilia was 29% and 24% and hypergammaglobulinemia (IgE) was 2,570 and 1,518 IU/ml for cases 1 and 2, respectively.

To date, 39 cases of toxocarasis have been identified in Japan [3]. The frequency of toxocarasis, possibly caused by the ingestion of raw meat, seems to be increasing in Japanese adults. Five cases of toxocarasis were reported in the Kyushu area of Japan [5, 6], and all gave a history of ingesting raw hen or cow liver. Our patients also had a history of eating uncooked chicken. Before their present illness, they did not live or work in an environment with significant numbers of dogs or cats, nor had they eaten raw meat except for the uncooked chicken. However, on further questioning as to the source of the ingested chicken, we found that the cock had been kept in the garden of the patients’ uncle, together with several dogs. We sought but did not receive permission to examine the dogs. In addition, several adults who dined with the patients (father, uncle, friend) complained of the same clinical symptoms. It seems likely the patients acquired their parasites from *T. canis*-contaminated raw chicken giblets.

The larvae in the raw chicken probably originated from eggs passed in the feces of domestic or stray cats. This speculation was given additional support by a recent report [7]: After *T. canis* eggs were injected into the gizzard of chicks, larvae were later found in the liver and were capable of infecting rats. This suggests that cats can serve as paratenic hosts for *T. canis* and that the host specificity of the ascarid is broader than previously thought. The natural hosts of *T. canis* (dogs and sometimes cats) are infected either by ingestion of infective eggs or transplacentally. About 20% of the dogs and cats of Kanagawa Prefecture in Japan were found to be infected with *Toxocara* species [8], suggesting that many domestic chickens could also be contaminated with the parasites.

It has become common for families or individuals to keep dogs or cats as pets. The high frequency in toddlers is undoubtedly due to the placing of objects (e.g., toys, utensils, fingers) contaminated with pet excreta into the mouth. In addition, adults must now be aware of the danger of ingesting raw chicken.