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Helicobacter Infection in Laboratory Mice: History, Significance, Detection and Management

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History

Since *Helicobacter pylori* was first isolated, from humans, in 1983, a number of *Helicobacter* species have been identified in domestic and laboratory animals. The first *Helicobacter* found in laboratory rodents was *H. muridarum*, reported in rats and mice in 1992 (11). The animals from which the organism was isolated had no clinical signs. *Flexispira rappini*, now called *H. rappini*, was reported in 1993 (13), also from mice with no clinical signs. *H. hepaticus* was first reported in the peer-reviewed literature in 1994 (10), although an abstract (14) and letter (4) had circulated in the preceding months. Even more recently, yet another *Helicobacter*, *H. bilis*, has been reported (8,9). The discovery of numerous *Helicobacter* spp. in mice has paralleled the description of numerous *Helicobacter* spp. in other mammals, and it is probably reasonable to think additional "new" *Helicobacter* spp. will be described in the future.

Biology and Significance

In the decade since *H. pylori* was identified as an etiologic agent of recurrent gastric ulcers in humans, numerous papers have appeared describing animal models of human *Helicobacter* gastritis, including both natural and experimental infections (1-3,5,6).

Of the *Helicobacter* spp. which colonize the gastrointestinal tract of mice, only *H. hepaticus* has been reportedly used in pure culture to reproduce natural disease, fulfilling Koch's postulates (15,16). Various strains of mice were reported to differ greatly in susceptibility to *Helicobacter*-induced disease, with A/JCr mice being highly susceptible and C57BL mice being relatively resistant, despite persistent infection in both strains. In addition to developing chronic hepatitis, infected A/JCr also developed a high incidence of liver tumors. Clearly, however, when it comes to *Helicobacter*, infection cannot be equated with disease.

Of the other *Helicobacter* spp. of mice, *H. rappini* has not been associated with lesions in mice, *H. muridarum* has been observed in gastric glands of mice with chronic gastritis, and *H. bilis* has been reported in the livers of mice with chronic hepatitis (9). For neither of the latter organisms, does the peer-reviewed literature contain a report of Koch's postulates being fulfilled.

In general, it is widely believed that *H. hepaticus* is a primary pathogen, but the pathogenicity of the other *Helicobacter* spp. is not convincingly demonstrated. The question of the potential pathogenicity of the other murine *Helicobacter* species will undoubtedly be vigorously explored in the next few years, both to answer concerns about the research impact of the organisms and to search for new animal models of human *Helicobacter* disease.

Detection

There are currently three widely used methods of detecting *Helicobacter* infection, each with some drawbacks. The original method of diagnosis, using a Steiner modification of the Warthin-Starry silver stain to directly observe spiral bacteria in bile canaliculi, is considered diagnostic. However, histologic differentiation among *Helicobacter* spp. is virtually impossible (low specificity). Histology is also notoriously insensitive, not only because large numbers of bacteria must be present in order for them to be observed in a microscopic section, but also because the bacteria may be present in the liver only late in the course of infection and, even then, only in susceptible strains of mice. Unfortunately, observation of spirochetes in the intestine is of no diagnostic significance since unrelated but morphologically similar spirochetes are part of the basic flora of even gnotobiotic mice. Electron microscopy can differentiate among the murine *Helicobacter* spp. described

to date, and is used on tissue samples or cultural isolates, primarily the latter. Electron microscopy, however, is very expensive, time consuming, and requires a sample with a high number of organisms.

Microbiologic culture of fecal pellets or cecal smears may also be used to isolate *Helicobacter* spp. (7,12,13) from laboratory mice. However, the technique is difficult, requiring special media and microaerophilic culture conditions. In addition, the sensitivity is not established, and no conclusive biochemical means of differentiating among the murine *Helicobacter* spp. has been described. For example, in order to select for the slow-growing *Helicobacter*, a fecal or cecal suspension is passed through a 0.45 micron filter to remove most other bacteria and particles. This technique increases the sensitivity of the culture method for *H. hepaticus*, but may actually remove *H. bilis* and other *Helicobacter* spp. The filtrate is inoculated onto *Brucella* agar with horse blood and TVP (trimethoprim, vancomycin, polymyxin B). The currently identified murine *Helicobacter* species, *H. hepaticu*s, *H. bilis*, *H. muridarum*, and *H. rappini*, are all gram negative, motile, helical bacteria and all are urease positive.

Polymerase Chain Reaction (PCR) is currently the most rapid and perhaps the most sensitive method of diagnosis for *H. hepaticus*. Recently, use of restriction endonucleases has permitted differentiation between *H. hepaticus*, *H. bilis*, and *H. muridarum*. PCR for *Helicobacter* spp. is conducted on fecal pellets, cecal contents or liver homogenates, and examines for the DNA of the organism.

With all of the diagnostic methods discussed here, the organism must be present to be detected, mere exposure will not give positive results. Development of a serologic test for antibodies specific for each of the various *Helicobacter* spp. would provide means of rapidly screening for animals which have been exposed to the organism, although for some bacteria antibodies are produced only if invasion, not just colonization, occurs. This might potentially limit the sensitivity of *Helicobacter* serology, as invasion of the intestinal tissue may only occur in older individuals of certain strains of mice.

At the current time, PCR of contents of the cecum or colon or fecal pellets, in combination with culture is recommended as the most comprehensive method of screening for *Helicobacter* infection.

Management

For many diseases, experience allows us to make good estimates of the number of animals which must be tested, and of what age, sex and strain, in order for negative results (finding no positives) to confirm that the colony is free of that particular disease. Unfortunately, these test parameters are not known for *Helicobacter*. However, in colonies infected with *H. hepaticus*, the prevalence of positive results by both PCR and culture appears to be high. In these colonies, lesions are more frequent in males than in females, and lesions increase in incidence after the animals are 6 months of age. In contrast to differences in disease susceptibility, essentially all ages and sexes seem equally susceptible to infection. In infected colonies, *Helicobacter* can be readily detected even in strains of mice which are resistant to *Helicobacter*-induced disease. With regard to how many mice to test, until more specific information is available, testing of 8 -10 animals is recommended.

Charles River has tested all mouse colonies by both culture and PCR of cecal contents for *Helicobacter hepaticus*. The special culture medium and incubation conditions used will support growth of all of the *Helicbacter* species which have been reported in mice, although the filtration step is specifically designed to increase the sensitivity of the technique for *H. hepaticus*. In addition, suspicious liver lesions will continue to be examined with a silver stain. All CRL mouse colonies in North America and Japan have been tested, and testing of CRL mouse colonies in other parts of the world will be concluded shortly. All tested colonies are negative for *H. hepaticus*. Results, updated weekly, are available on the **Charles River Home Page at http://www.criver.com**, and are also included in all CRL health reports. Should any CRL colonies be determined to be positive for any pathogenic *Helicobacter* species, we will move expeditiously to contain and eliminate the infection. At the same time, we will endeavor to avoid disrupting the supply of animals to informed customers whose particular research might not be affected by the presence of *Helicobacter* spp.

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