Helicobacter sp. Detection in Taconic Germantown, New York
Murine Pathogen Free™ Barrier IBU22
Updated 7/24/09

Routine IHMS-52 health testing of fecal samples collected from the BN/MolTac rat colony housed in IBU22 were PCR positive for *Helicobacter* sp. on June 23, 2009**.** The DNA was immediately sent to an outside laboratory on June 23, 2009 for additional testing and was reported as positive for *Helicobacter* sp. on June 26, 2009. Concurrently, 15 pooled fecal samples from 45 rat cages were sent from IBU22 on June 24, 2009 for additional testing. These samples were positive for *Helicobacter* sp. on June 29, 2009. RFLP analysis of the PCR product from the routine health testing analysis from the outside reference laboratory indicated that the DNA was not from *H. hepaticus*, *H. bilis*, *H. rodentium*, *H. trogontum* or *H. typhlonius*. Distribution of animals from the barrier was halted on June 30, 2009 while additional confirmatory testing and investigation into this atypical Helicobacter occurred. Concurrently DNA sequence analysis was performed on the original PCR amplicon. On July 6, 2009 the 709 bp amplicon was sequenced and was found to be 96% identical to *Helicobacter pullorum* sequences. As noted below, this is a novel finding in rodents that we are investigating.

Subsequently, both live animals and pooled fecal samples from mice were sent to an external reference diagnostic laboratory.

Results from these samples were reported on July 2, 2009 as follows:

Live BN/MolTac rats 21 /30 fecal PCR positive for *Helicobacter* sp.*
Pooled mouse feces[#] 24 /30 fecal PCR positive for *Helicobacter* sp.*

* Further analyses confirmed that the amplicon was not *H. hepaticus*, *H. bilis*, *H. rodentium*, *H. trogontum* or *H. typhlonius*

[#] Each pooled fecal sample represented 3 cages. Pooled samples were collected from C57BL/6NTac, C3H/HeNTac and DBA/2NTac. Pooled mouse fecal samples from each line and from each room were found positive.

Three of the above rat PCR amplicons and three of the pooled fecal amplicons were then sequenced by the external reference laboratory and reported on July 10, 2009 as *H. pullorum* with a sequence identity of 98%.

On June 30, 2009 gross necropsies were performed on 50 random C57BL/6NTac for cryopreservation of select tissues. Some mice had minimal amount of red tinged peritoneal fluid. All other abdominal viscera appeared normal.

Based on the gross findings on June 30, an additional 10 C57BL/6NTac mice were euthanized on July 2, 2009 and samples collected for histopathologic and pertineal fluid analyses. Results are pending.

IBU22 is a single building that has three animal rooms which share personnel and often moves animals between rooms for production purposes. The HVAC is separate and distinct from all other barrier locations. Room 1 produces C57BL/6NTac mice, Room 2 produces DBA/2NTac and C3H/HeNTac mice and BN/MolTac rats, and Room 3 produces C57BL/6NTac mice. Use of these mice and rats to fill customer orders was suspended from all three rooms as of June 30th.

Recent test results:

Routine IHMS™ Testing:
Taconic's routine International Health Monitoring System (IHMS™) testing screens for ***Helicobacter sp.*** every 13 weeks and results are posted on Taconic's website. Recent test dates and results specifically for ***Helicobacter sp.*** in IBU22 are:

Sample Date	Panel Type	Results for *Helicobacter. sp.*
6/16/09	IHMS-52	5/10
3/10/09	IHMS-13	0/9
12/16/08	IHMS-26	0/9

During the testing described above no clinical, gross or histopathologic observations suggestive of infection with *Helicobacter sp.* were made**.**

Treatment of IBU22 animals

At this time we have not determined how *Helicobacter pullorum* entered IBU 22. Entry via technicians working in the barrier is a strong possibility as is any animal movements into the barrier. All animal movements in to IBU 22 are from proven Defined Flora gnotobiotic isolators housing inbred foundation colonies. Technicians are assigned to IBU 22 solely and do not move to other barrier units until they undergo a 3 day quarantine. We suspect that entry via personnel is the highest probably route of entry but can not confirm this.

While in quarantine, all caging materials leaving IBU 22 are placed in stainless steel containers and autoclaved prior to processing. All animal movements out of the barrier have been halted with the exception of those requiring humane euthanasia. Animal care staff were required to go directly from the barrier to their homes and not associate with any personnel on site.

Who we are contacting and why?

Our commitment is to provide customers with complete, accurate and timely information on the research animals used in your studies. As part of that commitment, Taconic provides immediate and full disclosure of any events that may impact your research studies. This includes notification of all clients who received animals from a Barrier Unit whenever we detect any microbiological agent that is not accepted under the health standard established for that Barrier Unit. In this case we are contacting anyone receiving animals from IBU22 since March 10, 2009, which is the date of the last negative result.

What is the potential impact to my facility?

Introduction of *H. pullorum* into colonies may complicate health test result interpretation for Helicobacters. *H. pullorum* is detected by Helicobacter genus PCR assays and in order to differentiate *H. pullorum* from other Helicobacters additional testing is required.

Status of Helicobacter in other Taconic Barriers

Taconic has begun heightened testing for those areas in Germantown with human and animal traffic overlap from IBU22. As of July 22, 2009, one additional location, MBU3B, is suspect for *Helicobacter sp.* Taconic is in the process of determining the species at this time. We must advise you that animals in MBU 3B are suspect positive for *Helicobacter species.* We can confirm this is a *Helicobacter species* and that it is **NOT** a rodent helicobacter such as *H. hepaticus, H. bilis, H. rodentium, H. typhlonius or H. trigontum*. We believe that if the suspect is confirmed positive the species is most likely to be *helicobacter pullorum*. Currently, additional sampling and testing are underway to confirm both the positive *Helicobacter* species and to conclusively identify the species of *helicobacter* we detected the decision to receive animals from MBU3B is at your discretion. Taconic's Veterinary Services staff would be pleased to give you any additional information you require.

Epidemiologic Investigation

A thorough and systematic approach to investigate all possible sources and routes of entry of this organism is in progress. As these data become available updates will be provided.

What is *Helicobacter pullorum?*

Helicobacter pullorum, an enterohepatic Helicobacter species, has been isolated from feces of avian species and humans. There appear to be no published reports of natural infection of rodent species by *H. pullorum* nor are we aware there are published experimental infection studies of *H. pullorum* in rodents. The source of the *H. pullorum* in the barrier and route of introduction is unknown at this time. Based on other Helicobacters, a fecal-oral transfer route is presumed. There have been reports of an association of *H. pullorum* in people with inflammatory bowel disease, diarrhea, and other gastrointestinal symptoms. However, there have also been reports of *H. pullorum* being present in the intestinal tract of people with no clinical signs or symptoms.

References:

Aliment Pharmacol Ther. 2009 May 5.
Association between entero-hepatic Helicobacter species and Crohn's disease: a prospective cross-sectional study.Laharie D, Asencio C, Asselineau J, Bulois P, Bourreille A, Moreau J, Bonjean P, Lamarque D, Pariente A, Soulé JC, Charachon A, Coffin B, Perez P, Mégraud F, Zerbib F.

Gut. 2009 May;58(5):629-35. 2008 Jun 25.
Study of Helicobacter pullorum proinflammatory properties on human epithelial cells in vitro.Varon C, Duriez A, Lehours P, Ménard A, Layé S, Zerbib F, Mégraud F, Laharie D.

Int Microbiol. 2008 Sep;11(3):203-8.
A novel real-time PCR assay for the detection of Helicobacter pullorum-like organisms in chicken products.González A, Piqueres P, Moreno Y, Cañigral I, Owen RJ, Hernández J, Ferrús MA.

Prevalence of Helicobacter pullorum among patients with gastrointestinal disease and clinically healthy persons.
Ceelen L, Decostere A, Verschraegen G, Ducatelle R, Haesebrouck F.
J Clin Microbiol. 2005 Jun;43(6):2984-6.

Vet Microbiol. 2006 Mar 10;113(1-2):45-53. 2005 Nov 28.
The cytolethal distending toxin among Helicobacter pullorum strains from human and poultry origin.Ceelen LM, Haesebrouck F, Favoreel H, Ducatelle R, Decostere A.

Cytolethal distending toxin in avian and human isolates of Helicobacter pullorum.
Young VB, Chien CC, Knox KA, Taylor NS, Schauer DB, Fox JG.
J Infect Dis. 2000 Aug;182(2):620-3. Epub 2000 Jul 19.

J Morphol. 2009 Aug;270(8):921-8.
Mitotic catastrophe as a prestage to necrosis in mouse liver cells treated with Helicobacter pullorum sonicates.Ceelen LM, Haesebrouck F, D'Herde K, Krysko DV, Favoreel H, Vandenabeele P, Ducatelle R, Decostere A.

For additional information please contact a member of Taconic's Veterinary Services at 1-888-822-6642 or your Taconic Area Technical Representative.