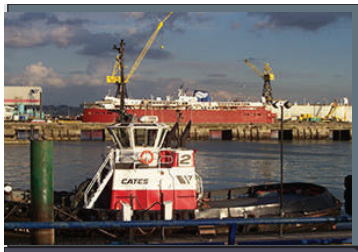

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CMPT QUARTERLY ON-LINE NEWSLETTER

Volume 14 Number 4

INSIDE THIS ISSUE

Coordinator's report 2009 -2010

by Esther Kwok

ISO 9001:2008

QMI-SAI Global conducted a re-assessment audit on our quality management system against the updated standard, ISO 9001:2008, on May 7, 2010 and found that CMPT had met all its requirements.

The auditor found three opportunities for improvement – 2 of which we've resolved. The third was a suggestion regarding access to the website. The auditor indicated that the intellectual property, such as the critiques available on the web, www.cmpt.ca, were valuable and that they should only be available to members and registered labs. At the moment, we're looking into a members' only site where existing registered labs can have access. The goal is to have a site that can be entered by non-registered parties also, for which we will have to investigate further.

Finances

We will have to increase our program fees substantially for 2011-2012 due to the increase in expenses and decrease in enrollment in 2010-2011. Additionally, over the last couple of years, we've increased value to our programs, particularly the Clinical Microbiology program, with new and more complicated challenges and feel that the added value should be reflected in the fees.

Clinical Microbiology

The Gram Smear Supplementary Program has had a full year's run and it's been very successful, more so than we had anticipated. Currently, the program has 82 participants, representing 63% of our participants. Many of the labs are using the program to enhance the technical expertise of their staff, particularly those of the off-shift staff when required to read stat Gram smears.

Water Bacteriology

Last year, we had reorganized the water program and divided the program into two programs, the drinking water and the recreational water program. Individual recreational water samples were available to the labs. Although this was beneficial to the labs, it was logistically challenging for us to monitor the specific needs of each laboratory. For that reason, the individual recreational water samples will no longer be available in the 2011-2012 program year, and only sets of three recreational samples will be available.

Parasitology

It has been no secret that for the last couple of years, we've had a difficult time obtaining Parasitology samples. The cost of the material continues to be quite expensive. We are looking into alternate sources of material.

Thanks again to BCCDC and BC Biomedical Labs for their technical support and assistance this past year.

We have had an international participant in the past, Bangladesh in our water program and we have had interest this year from the United Arab Emirates in the *C.difficile* program and Malaysia in molecular samples. But, this year, the CMPT Parasitology program has gone international. We would like to welcome Sweden to the Parasitology program.

Mycology

Welcome Dr. Romina Reyes of LifeLabs and Dr. Jeff Fuller of the University of Alberta to the Mycology committee.

cIQc

Beginning in 2010, CMPT began collaborating on cIQc's EQA program for breast tumor markers. cIQc is the Canadian Immunochemistry Quality Control program which provides samples in ER, PR, Her2 (estrogen & progesterone receptor proteins and the human epidermal growth factor receptor2), IHC and FISH. Originally established at the University of Saskatchewan and founded by Dr. Emina Torkalovic and Dr. Blake Gilks, it is now established at the University of Toronto and administered by UBC's Department of Pathology. With participants across Canada, including Quebec and Ontario and internationally, including labs in the US, Denmark and Brazil, it is currently one of two EQA programs available in Canada. The program was made available in April 2009 and is supported by the Canadian Partnership against Cancer. CMPT's main role is to provide quality management, administration, logistics, shipping and handling.

COORDINATOR'S REPORT

International Delegates

This year, we welcomed two groups for proficiency testing training, Oman, and South Africa.

This Spring, the World Health Organization sponsored two delegates, Aisha Salim Al-Jaaidi and Mohamed Khudadat Al-Bulushi, from the Central Public Health Laboratory, Ministry of Health, Sultanate of Oman for training. Their EQA program ships to 26 countries in Middle East and four reference labs, which include the United Kingdom and South Africa.They spent two weeks at CMPT for PT product manufacturing, quality management, and administration.

This past July, Dr. Olga Perovic, Vivian Fensham, and Bhavani Poonsamy from Microbiology External Quality Assessment Reference Unit in Johannesburg, South Africa spent one week doing a PT refresher at CMPT. Their program administers EQA to countries in Sub Saharan Africa

The Future

Future projects and product development we're looking into is mainly in Molecular PT.

We surveyed laboratories last spring to see if there would be interest in molecular testing. We sent a list of possible testing organisms, methicillin resistant *S. aureus* (MRSA), vancomycin resistant *Enterococcus* (VRE), group B *Streptococcus* (GBS), *Neisseria gonorrhoeae* (GC), *B.pertussis* & *B.parapertussis,* Shiga toxin, *C. difficile, Mycoplasma* and *Chlamydia trachomatis* and asked participants if they would be interested in receiving molecular testing PT samples if they were made available. Of 94 responses, 21 indicated they wanted GC, 24 indicated they wanted *C.trachomatis*. We're looking into the details on the production of these samples.

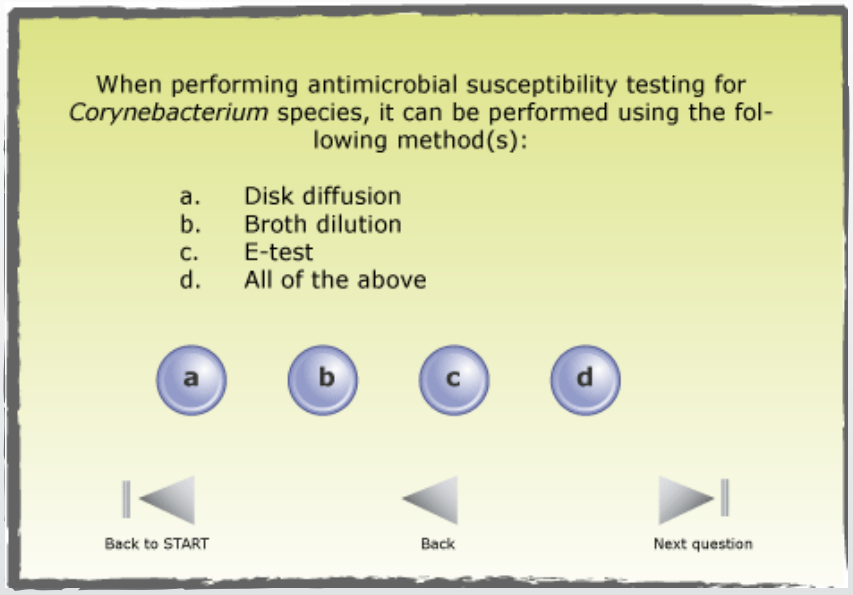
The idea would be to create generic samples that labs could place into any of their transport media of any commercial system. We're at the beginning of the product development and will have more information soon.

Although only 10 and 7 labs, respectively, indicated they would be interested in the MRSA and VRE molecular testing, we will be offering the samples with next year's programs.

With Appreciation

CMPT staff & committee members, UBC Department of Pathology, POLQM, cIQc, Accreditation Programs, Vancouver Coastal Health Authority, Vancouver General Hospital Microbiology Laboratory, BCCDC Environmental, General Bacteriology and Parasitology Labs, EWQA (Enhanced Water Quality Assurance), BC Biomedical Labs, University of Alberta Hospital, Microbiology and Mycology Laboratories, Queen Elizabeth II Hospital Microbiology & Environmental Services in Halifax, BC Ministry of Environment, MacLean Centre for Tropical Diseases at McGill University, UBC Farm, VWR and especially, all the participating laboratories.

DOWN MEMORY LANE



Strategies for Diagnosis and Management of *Clostridium difficile* Infections

R. P. Rennie, Laboratory Medicine and Pathology, University of Alberta, Edmonton, Alberta

Introduction

Disease caused by *Clostridium difficile* is related to acquisition of *C. difficile* in the gastrointestinal tract, alteration of normal gut flora, and production of toxins. Three known toxins, A (an enterotoxin known as tcdA), B (a cytolytic toxin –known as tcdB) and a newly recognized binary toxin are responsible for the clinical symptoms of infection. *C. difficile* infection (CDI) may either be related to antimicrobial use (antimicrobial-associated diarrhoea or AAD) or primary pseudomembranous colitis with AAD as a contributing factor. Clindamycin and beta-lactams (usually cephalosporins) are the most common agents involved, but most antimicrobials have been implicated.

Clinical and pathologic observations

Pseudomembranous colitis (PMC) is identified by colonoscopy. The pathology is identified as adherent plaques, 2 to 10mm in diameter which may be either patchy or confluent (Figure 1). Under the microscope, PMC is identified by the volcano-like appearance of the cellular inflammatory response to the toxins produced by *C. difficile* (Figure 2). The identification of PMC has good specificity for CDI but relatively poor sensitivity. PMC is often absent early in the infection process and is often absent in AAD. Endoscopy should be reserved for emergency cases, in patients with severe colitis, or those with persistent diarrhea when other causes have been ruled out.

Outbreaks and Economics

In recent years an increased severity of CDI has been observed in some patients. Some strains of *C. difficile*, notably a new genotype NAP1/O27, toxinotype III has been shown to produce 20-fold greater concentrations of toxins A and B during logarithmic phase growth (Warny 2005). This is due to an 18 base pair deletion in the tcdC gene that regulates toxin production. Many of these NAP 1 strains also contain a binary toxin and fluoroquinolone resistance in common. These strains are problematic in an outbreak situation since typing methods (e.g. Pulsed Field Gel Electrophoresis – PFGE-) may not be able to distinguish sufficiently between strains.

The costs of CDI are potential enormous. Data for both the United States and the United Kingdom suggest that excess length of hospital stay due to CDI may be as high as 15 – 17 days, depending on ward, intensive care requirements, and whether the infection was acquired in hospital. Excess costs may be anywhere from $5000 to $25,000 per patient depending on the specific patient and infection with a NAP 1 strain.

The total costs based on an incidence of 7 per 10,000 population (UK data) are in excess of $200 million annually with a total excess hospital length of stay of about 750,000 days. Ancillary costs related to mortality, disability and productivity are completely unknown.

The two most notable recent outbreaks of CDI in Pittsburgh in 2000 (Muto , 2005) and Quebec in 2004, showed that there was a doubling of severe life threatening disease (1.6 – 3.2%) with attributable mortalities of up to 7% (30 day) and 17% (one year). For those more elderly persons the impact on mortality increased up to 10 – 15%. Using data from the Quebec outbreak, in 2010, Miller et al showed that there was a significant association between the strain type (i.e. NAP 1), and age-related death. Risk factors for relapse are important also from a diagnostic perspective. Age, fever, concomitant antimicrobials, severe or fulminant infection, and laboratory findings of increased white count, increased albumin, and the presence of NAP1 strains are important observations (Gravel et al 2010).

Treatment of CDI

Primary therapy has been metronidazole or oral vancomycin in mild to moderate toxin-positive cases. In severe cases these agents have been combined. Other therapies have included vancomycin enemas, infusion vancomycin into a cecal catheter, bacitracin, and replacement therapy including yogurt (lactobacilli) and fecal transplant. There are a number of newer therapeutic modalities, including fidaxomicin (Gerding, 2010; Tannock 2010), intravenous immunoglobulin (IVIG) and monoclonal antibodies (Lowy, 2010) to tcdA and tcdB, but there is not sufficient data yet to confirm their reliability.

Laboratory diagnosis

For accurate diagnosis only diarrheal stools should be collected. The Bristol stool chart (Lewis, 1997) is helpful in determining if a feces sample is diarrheal. Rectal swabs or biopsies are not recommended. The stool should be held at 4^{O}C before processing. There is little evidence available regarding interference with barium or concurrent antimicrobial therapy for other infectious processes. There is poor correlation of fecal white count and infection.

Older methods such as counterimmunoelectrophoresis (Rennie, 1984) have now been supplanted by a number of new laboratory tools now available for relatively rapid diagnosis. These include examination for *C. difficile* products (eg.glutamate dehydrogenase- GDH), culture to identify toxigenic strains, cytotoxicity of stool filtrates, enyzyme immunoassay against tcdA and tcdB, and RT-PCR. In the presence of appropriate clinical history each of these examina-

Figure 1. Pseudomembranous colitis

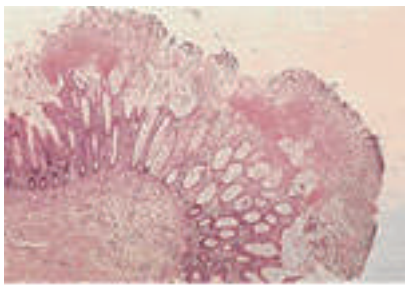


Figure 2. Cellular inflammatory response

tions has the potential to provide a positive result. Serology has not been shown of value for accurate diagnosis.

Glutamate dehydrogenase is a metabolic enzyme produced by *C. difficile*. The test is available from a small number of manufacturers. It is usually provided in the form of a solid-phase line immunoassay and is rapid and easy to perform. It has been shown to have sensitivities approximating 90% (Crobach 2009) and appears to be excellent as a negative screening method (Ticehurst 2006). However, when used alone, this method has a low positive predictive value (PPV) and cannot determine if the causal strain is toxigenic or not.

Toxigenic culture involves growth on selective media such as cycloserine cefoxitin fructose agar (CCFA) (George 1979) followed by in vitro toxin detection. Some samples require ethanol or heat shock to enhance culture depending on the organism load. In vitro toxin detection may be performed by cytotoxin assay from broth culture supernates incubated for 2 – 5 days, by EIA either from a broth supernate or direct colony suspension, or by PCR. Direct EIA from colony suspension has not been fully validated, but Lalande (ECCMID 2010) reported that the method was very sensitive to identify toxigenic strains. The difficulty with direct culture is the delay in confirmation of a positive result.

Enzyme immunoassay of fecal filtrates has been the most widely utilized method for many years. The more recent tests identify both tcdA and tcdB. In a 96 well format or in a solid phase format, many samples can be tested at the same time, and turn-around is within the same day. The issue of tcdA negative, tcdB positive strains which may occur in up to 10% of samples (depending on study) (Alfa, 2000) is solved by the combination EIA. The sensitivity of EIA is however, potentially problematic. Compared to either cytotoxic assay or toxigenic culture, EIA sensitivity is anywhere from 60 – 80%. However, the specificity is still high (Crobach 2009; Planche 2008).

Recent advances in molecular diagnostics using a variety of probe technologies (FRET, Taqman, Sybergreen , or molecular beacon) have shown sensitivities of 85 to 95% with high. In these studies analytical sensitivities have still shown that at least 104 CFU per gram of stool is required for a positive probe (Sloan, 2008, van den Berg, 2007; Peterson, 2007; Belanger, 2003). A number of commercial manufacturers have entered this area with the target being primarily tcdB since almost all strains have the tcdB gene. The methods are either manual or automated. Processing time for all these new systems is approximately 3 hours. Compared to cytotoxic assay or toxigenic culture, the sensitivity is anywhere from 75 – 95% depending on the prevalence of infection in the sample studied. Indeterminate results may be as high as 7% (Stamper 2009; Kvach 2010; Terhes 2009; Huang 2009; Novak-Weekley 2010). The advantages of molecular testing are rapid turn around, high sensitivity, and the binary toxin and tcdC deletion (a surrogate marker of the hypervirulent strain O27 can be detected. The disadvantages are that the probe detects the gene, not the toxin The tcdB gene has been shown to drift and other hypervirulent clones (e.g. O78/126) have been observed.

The various new methods have been recently compared (Eastwood et al, 2009) on over 600 diarrheal stools. Using toxigenic culture as a gold standard, the mean sensitivity and specificity of the toxin detection assays were 75 and 96% respectively. The GDH assay had a sensitivity and specificity of 88 and 94% respectively. At a 10% prevalence, the PPV was low 50 – 90% compared to toxigenic culture.

Based on all these data, with acknowledgement of the importance of rapid turn around to prevent spread of *C.difficile* in hospital units, a three step algorithm (Figure 3,) has been proposed by Schmidt and Gilligan (2009) and Verdoorn (2010).

The sample would first be screened with a combined GDH/tcdA-tcdB EIA test.

These are now available commercially in a solid phase line assay format. If the combined test is negative then the patient does not have *C. difficile* infection. If both are positive then the patient is presumed to have CDI. If only the GDH is positive, then the sample should be tested by PCR. A positive PCR is indicative of infection (despite the fact that the EIA for tcdA and/or tcdB is negative). A negative PCR indicates that the patient is most likely carrying a non-toxigenic *C. difficile* strain, and other sources for the diarrhea should be investigated. PCR may also be useful in suspected outbreaks with hypervirulent strains to study the epidemiology and help direct infection prevention and control strategies.

There are two additional issues to consider. The first is re-testing. Two recent studies (Aichinger, 2008; Cardona, 2008) have shown that there is little advantage to repeat testing within a seven day period. Increases to approximately 10% were observed if re-tests were done greater than seven days after an initial result in the face of continuing diarrhea. The second issue is co-infection with another enteric pathogen. Both personal experience and evidence in the literature indicates that other pathogens present in feces may be either missed or lead to increased severity of illness if diagnostic efforts are focussed only on *C. difficile* (Simmonds SD, 1987)

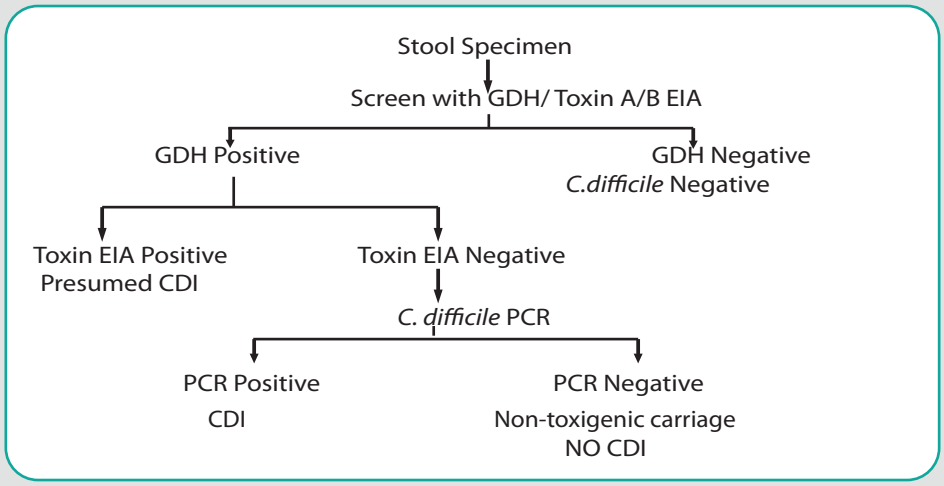


Figure 3. Algorithm for microbiological investigation of *C. difficile* infection

Summary

The world of *C. difficile* infection has changed! In some places increased prevalence of NAP1/O27 strains has resulted in increased severity, morbidity and mortality. There are a number of novel treatment options are on the horizon but these still require further testing and validation. It is clear that diagnostic laboratory methods may be variable, particularly in low prevalence populations. A multi-step approach is required for routine diagnosis of infection and even then not all patients will be captured. What is important is that the approach should be available close to hospital in-patients due to rapid deterioration of antigens and toxin if samples are not stored properly (Johnson, 2007). More sophisticated reference laboratory capabilities (PCR) can be used to confirm difficult cases, and to monitor the epidemiology of more severe cases caused by hypervirulent strains of *C. difficile*. Last but certainly not least, infection prevention and control is an integral component of the strategy, requiring good communication, heightened awareness and laboratory support.

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Leptospira and leptospirosis

Paul N. Levett PhD, (D)ABMM, FCCM, FAAM Saskatchewan Disease Control Laboratory; Regina, Saskatchewan

The genus *Leptospira* is comprised of spiral-shaped bacteria with hooked ends (Figure 1). Leptospires are tightly coiled spirochaetes, usually 0.1 µm x 6-20 µm. Morphologically all leptospires are indistinguishable; viewed by dark-field microscopy, leptospires exhibit two distinct forms of movement, either translational (rapid back and forth movements) or rotational (spinning rapidly about the long axis of the cell).[1] Leptospires are obligate aerobes, with an optimum growth temperature of 28-30^{O}C. They grow in simple media but require long chain fatty acids and vitamins for growth.[2]

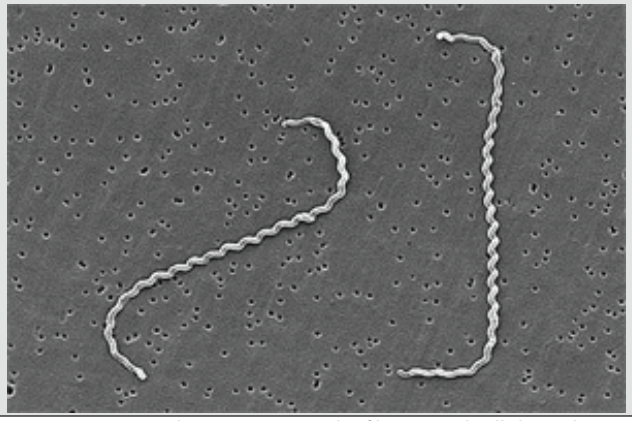

Figure 1. Scanning electron micrograph of leptospiral cells bound to a 0.2 µm filter. Magnification, approximately x 3,500. (Public Health Image Library, CDC).

Historically, the leptospires were classified into one of two species, Leptospira interrogans, representing the pathogenic strains isolated from man and animals, and *L. biflexa*, representing the saprophytic strains isolated from water.[2] Both *Leptospira* species were divided into numerous serovars, defined by agglutination after cross-absorption with homologous antigen. Serovars that are antigenically related have traditionally been grouped into serogroups, primarily for the convenience of serologists.[3] While serogroups have no taxonomic standing, they are useful for epidemiological understanding. The phenotypic classification of leptospires has been replaced by a genotypic one, in which 16 species include all serovars of *Leptospira* spp. (Table 1 and Figure 2).

The availability of several leptospiral genomes has led to a rapid increase in understanding of pathogenesis, [4,5] but the disease process is still poorly understood. [6] The immunology of leptospirosis is complex. [7] Vaccines are widely used in livestock and dogs, but there are few human vaccines and they induce only limited protection.

The disease in maintained in nature by chronic renal infection of carrier animals, and is transmitted from animal to animal by exposure to infected urine. Humans become infected through either direct or indirect contact with the urine or body fluids of an infected animal, either directly or indirectly via water or damp soil. [3]

Table1: Species of *Leptospira*, organized by phylogenetic relationships (see Figure 1)

Species of pathogenic leptospires	Species of non-pathogenic leptospires
L. alexanderi	*L. biflexa*
L. borgpetersenii	*L. wolbachii*
L. interrogans	
L. kirschneri	Species that may contain pathogenic and non-pathogenic leptospires (intermediate group)
L. kmetyii	*L. broomii*
L. meyeri	*L. fainei*
L. noguchii	*L. inadai*
L. santarosai	*L. licerasiae*
L. weilii	*L. wolffii*

The incidence of infection is much higher in warm-climate countries than in temperate regions. This is due both to longer survival of leptospires in the environment in warm, humid conditions and to greater opportunities for human exposure. The incidence of leptospirosis is seasonal. It peaks in summer or fall in temperate regions, where the temperature is the limiting factor in the survival of leptospires. Leptospirosis also increases during rainy seasons in warm climate regions, where rapid desiccation would otherwise prevent survival of the organisms in the environment.

Leptospires enter the body through abrasions or cuts in the skin, or via the conjunctiva. Once introduced into the body, the

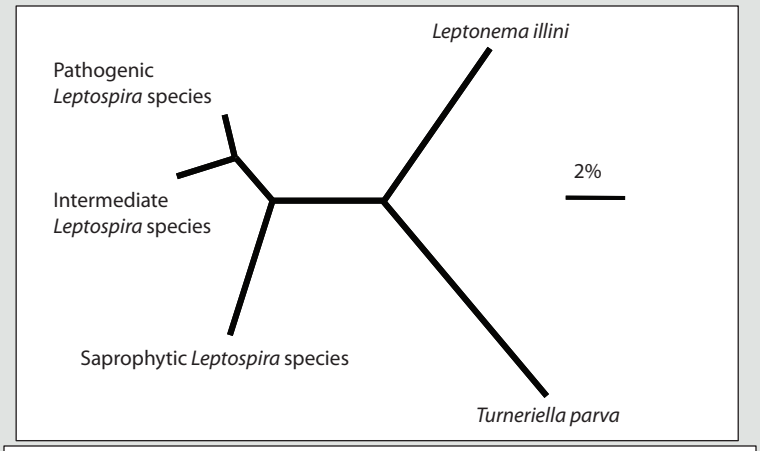

Figure 2. Phylogenetic relationships based on 16S rRNA gene sequences between the genera of the Leptospiraceae. Scale bar: 2 % difference in sequence.

leptospires multiply in the blood. Most human exposures result in either asymptomatic seroconversion or a mild, flu-like illness, but in about 10% of cases, the leptospires leave the blood and enter the organs and tissues, particularly the liver, kidneys and muscles. A very small proportion of cases suffer from severe multi-system disease manifesting as renal failure, jaundice, meningitis, myocarditis and pulmonary haemorrhage, which has a mortality rate of up to 20% if untreated. [6]

Because of the requirement for specialized culture media, isolation of Leptospira from suspected cases is rarely feasible. Most cases are diagnosed by serology. [6] IgM antibodies are detectable 5-7 days after the onset of symptoms and persist for many months after recovery from the acute disease. Convalescent serum specimens should always be collected, for testing of acute and convalescent samples using the microscopic agglutination test (MAT). This test is only performed in specialized reference laboratories. In Canada, serological testing of human samples is available at the National Microbiology Laboratory in Winnipeg. Rapid diagnosis by detection of leptospiral DNA in blood (collected in an EDTA tube), serum, CSF or urine, using PCR is possible and facilitates the initiation of appropriate antibiotic therapy if treatment was not begun empirically. Mild cases of the disease respond rapidly to oral treatment with doxycycline or amoxycillin, but severe disease requires both systemic antibiotic therapy and other supportive measures. [8] In fatal cases, the diagnosis may be confirmed by immunohistochemical staining of tissue sections from affected organs. [6]

Animals, including man, can be divided into maintenance hosts or accidental (incidental) hosts. Animals may be maintenance hosts of some serovars but incidental hosts of others, infection with which may cause severe or fatal disease. The most important maintenance hosts are small feral mammals, which may transmit infection to domestic farm animals, dogs and man. There is some degree of host-adaptation. Different rodent species may be reservoirs of distinct serovars, but rats are generally maintenance hosts of the related serovars Icterohaemorrhagiae or Copenhageni, and mice of serovar Ballum. Domestic animals are also maintenance hosts; dairy cattle may harbor serovars Hardjo and Pomona, pigs may harbor serovar Pomona, Tarassovi or Bratislava, and dogs may harbor serovar Canicola. Distinct variations in maintenance hosts and the serovars they carry occur throughout the world. Knowledge of the prevalent serovars and their maintenance hosts is essential in understanding the epidemiology and implementing control of the disease in any region.

The epidemiology of leptospirosis has changed significantly in recent years. In developed countries the disease is much less prevalent than it was a generation or two ago, for several reasons. Increasing urbanization has reduced the opportunity for exposure in the rural environment and immunization has largely controlled the disease in domestic animals. However, against this background there remain foci of infection, such as the recognition of continuing rat-associated leptospirosis in inner cities and the emergence of canine infection in Eastern North America caused by serovars Grippotyphosa and Pomona, [9] presumably acquired from racoons, skunks or possums.

There has been a significant rise in travel-associated leptospirosis, often associated with adventure tourism in the tropics, [10] and invariably associated with exposure to fresh water. Outbreaks of the disease acquired by athletes participating in triathlons [11] and adventure sports [12] have the potential for widespread media coverage, but by far the greatest epidemiological change globally has been the occurrence of large outbreaks of leptospirosis associated with excess rainfall. [13] These outbreaks often occur in regions where capacity for diagnostic testing is least well developed. The global burden of disease remains significant, and although leptospirosis is now a rare disease in developed countries, it is important that clinicians and laboratories retain the ability to recognize and diagnose infection in returning travelers.

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Editor: Veronica Restelli

Contact Connections:

By mail:
Room 328A, 2733 Heather Street,
Vancouver, BC
V5Z 1M9
Canada

By phone: 604-875-4685
By fax: 604-875-4100
By e-mail:
restelli@interchange.ubc.ca

Connections is available online:

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