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Review

A Primer on Novel Strains of *Echinococcus multilocularis* for Canadian Wildlife Biologists

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Abstract

Echinococcus multilocularis is the causative agent of alveolar echinococcosis, causing approximately 18,000 new cases each year. *E. multilocularis* is widespread with distinct strains in Europe, Asia, and North America. Recently, a mutant European type strain was detected in North America. This novel strain may be widespread in Canadian wildlife, infect domestic animals, and be the cause of recent human cases of alveolar echinococcosis in Canada. In this review, we cover the biology and diversity of *E. multilocularis*, the pathology and diagnosis of alveolar echinococcosis, and risk factors for exposure to invasive *E. multilocularis*.

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BACKGROUND

Tapeworms of the genus *Echinococcus* (Order Cyclophyllidea) are associated with zoonotic diseases including cystic and alveolar echinococcosis which impact over a million people (CDC 2019; WHO 2020). *Echinococcus multilocularis* is the causative agent of alveolar echinococcosis (AE), a potentially fatal infection associated with the liver (reviewed in Moro and Schantz 2008; WHO 2020). *E. multilocularis* may lead to ~18,000 new cases of AE worldwide annually and is considered a neglected disease by the World Health Organization (Torgerson *et al.* 2010; WHO 2020). However, different genotypes of *E. multilocularis* vary in pathogenicity and are, therefore, of different levels of concern from a public health perspective (Bartel *et al.* 1992; Torgerson *et al.* 2010).

A European-like strain of *E. multilocularis* has been detected in Canada as well as the USA — potentially introduced through importation of infected animals — and is associated with most Albertan cases of human AE (Gesy *et al.* 2013; Massolo et al 2019; Polish *et al.* 2020). *E. multilocularis* is widespread in Alberta, including urban centers (Luong *et al.* 2018; Massolo *et al.* 2019). Genetic analysis by Massolo *et al.* (2019) of Canadian samples revealed the presence of 3 new *E. multilocularis* strains possibly resulting from mutations of an imported European strain. Thus, there is a need for multi-disciplinary study and monitoring of this parasite, in particular its ecology and presence in canid hosts (both wild and domestic). Dog owners, trappers, hunters, farmers, organic gardeners as well as professionals who work with animals are more likely to be exposed to *E. multilocularis* eggs creating a need for increased awareness (Stehr-Green *et al.* 1988; Deplazes et al 2004). This short review aims to provide a general overview of *E. multilocularis* biology, the recent research on invasive strains in Canada, and increase awareness of precautions that should be taken during work with risks of exposure to invasive *E. multilocularis*.

GENERAL BIOLOGY

Lifecycle and Internal Biology

The sylvatic definitive hosts of *E. multilocularis* are canids such as coyotes (*Canis latrans*), wolves (*Canis lupus*), and foxes (*Vulpes* spp.) (Schurer *et al.* 2014; Mihmanli *et al.* 2016; Hemphill *et al.* 2019). The intermediate hosts are rodents (Jenkins *et al.* 2012; Miller et al 2016; Avcioglu et al 2017). Humans can become dead-end intermediate hosts upon accidental ingestion of infectious eggs (Jenkins *et al.* 2012; Wu et al 2017; Hemphill *et al.* 2019). AE is caused by larval infiltrates within the internal organs of the intermediate host (Gesy *et al.* 2013; Mihmanli *et al.* 2016; Budke et al 2017; Hemphill *et al.* 2019).

Adult stages develop within the intestinal tract of definitive hosts, where gravid proglottids release eggs expelled with host feces (Mihmanli *et al.* 2016; Hemphill *et al.* 2019). Eggs can remain viable in the environment for up to 240 days at -18ºC, whereas extreme cold (< -83ºC) can kill eggs within days (Veit *et al.* 1995). Heat can also deactivate eggs; 65ºC treatment for 180 min rendered eggs nonviable (Federer *et al.* 2015). Once ingested by the intermediate host, eggs hatch in the stomach and release hooked oncospheres (Hemphill *et al.* 2019).

Oncospheres penetrate the intestinal epithelium, enter the circulatory system, and travel towards the liver (Hemphill *et al.* 2019). There they mature into proliferating metacestodes, which contain an internal metabolically active germinal layer and an external acellular laminated layer (Bulakcici *et al.* 2016; Hemphill *et al.* 2019). These layers form the tumor-like alveolar hydatid cysts composed of necrotic irregular cystic cavities lined by laminated membranes, sometimes without nucleated germinal membranes or protoscolices (Jura *et al.* 1998; Gesy *et al.* 2013; Mihmanli *et al.* 2016; Wang and Gottstein. 2016; Hemphill *et al.* 2019). The laminated membranes are often fragmented and are strongly positive by Periodic Acid Schiff (PAS) stain. These structures invade necrotic liver tissue eliciting a variable host response with occasional granulomatous reaction and focal calcification (Jura *et al.* 1998; Mihmanli *et al.* 2016; Hemphill *et al.* 2019).

Protoscoleces develop within alveolar cysts and are released when the carnivorous host consumes cysts during predation of infected intermediates (Hemphill *et al.* 2019). Protoscoleces evaginate after consumption to enable attachment to the intestinal epithelium, then develop into adult tapeworms (Hemphill *et al.* 2019).

Diversity and Range

E. multilocularis is a cosmopolitan species with diverse genotypes in Asia, Europe, and North America (Nakao *et al.* 2009; reviewed in Knapp *et al.* 2015). Perhaps unsurprisingly, the European and Asian clades are more closely related to each other than to the North American strain. The European and Asian clades diverged 37,000–60,000 yrs ago, whereas North American strains have been isolated for at least 75,000 yrs when the North American Clade underwent a major internal divide (Nakao *et al.* 2009). Prior to observation of a European genotype in North America, the primary overlap between major clades was in

the St. Lawrence Island (Bearing Sea) region, likely due to its geographic placement (Nakoe *et al.* 2009). The relatively low geographic dispersal of European *E. multilocularis* relative to Asian conspecifics (Nakeo *et al.* 2009) suggests that recent observations of European strains in North America are likely human facilitated.

Invasive Strains in Canada

In 2009, a domestic dog (*Canis lupus familiaris*) in British Columbia tested positive for an invasive European strain (Jenkins *et al.* 2012; Gesy *et al.* 2013). By 2013, *E. multilocularis* was detected in approximately one-third of sampled Albertan canids, including coyotes sampled in both Calgary and Edmonton (Catalano *et al.* 2012; Gesy *et al.* 2013). The first human case in Alberta was also reported in 2013 (Massolo *et al.* 2019). Six more human cases in Alberta were confirmed over the next 4 yrs, with patients' travel and exposure histories as well as genotyping strongly suggesting local acquisition of the disease (Massolo *et al.* 2019). Several more cases have been reported to Alberta Health since.

Sequence analysis of parasitic mitochondrial DNA revealed presence of 3 new *E. multilocularis* strains in animal hosts: ECA, EAB, and ESK (Massolo *et al.* 2019). These novel strains displayed unique single nucleotide polymorphisms (SNPs) in either or both the cob and cox1 genes (Massolo *et al.* 2019). ECA was found to be the most common strain of *E. multilocularis* in Alberta, infecting 66/77 local definitive hosts and 5/7 patients (Massolo *et al.* 2019).

Disease

Pathology, diagnosis, and treatment

AE primarily affects the liver (Jura *et al.* 1998; Mihmanli *et al.* 2016; Budke *et al.* 2017; Hemphill *et al.* 2019), although metastasis to other organs, such as the brain and lungs, has been reported in between 22–34% of patients (Kern et al 2003; Mihmanli *et al.* 2016). Due to the slow growth of alveolar cysts, infected individuals can remain asymptomatic for up to 10–15 yrs (Jura *et al.* 1998; Mihmanli *et al.* 2016; Hemphill *et al.* 2019); however, the disease progresses faster and is more severe in immunocompromised hosts. The mortality rate 10-years post infection is 90% (Bresson-Hadni et al 2003; Gesy *et al.* 2013; Wang and Gottstein. 2016). Symptoms are generally non-specific and are dependent on and proportional to the degree of liver involvement. They may include abdominal pain, jaundice, fever, and weight loss (Mihmanli *et al.* 2016; Hemphill *et al.* 2019). Due to the long asymptomatic period under-diagnosis is likely, and delayed diagnosis may result in higher patient morbidity and mortality.

Diagnosis is often incidental because of the prolonged asymptomatic period (reviewed in Brunetti *et al.* 2010; Aydinli *et al.* 2012; Mihmanli *et al.* 2016). Presumptive diagnosis can be made based on imaging and immunological assays. Ultrasound, CT, and MRI can detect hepatic lesions and determine the extent of proliferation (Hemphill *et al.* 2019). Serological tests include EIA screening using purified/recombinant antigens (such as Em2, Em18, EmVF) and have sensitivity and specificity approaching 100% when performed in combination with confirmatory Western Blot (ProvLab Alberta 2018; Hemphill *et al.* 2019). Although EIA results may remain positive years after infection resolution as antigens remain in inactive lesions (Mihmanli *et al.* 2016), Em18 antibodies can be used to assess viability of the parasite.

PCR targeting the *E. multilocularis*-specific nucleic acids in tissue biopsies can be used to corroborate diagnosis. In fact, positive serology, histopathology compatible with AE, and/or detection of *E. multilocularis* nucleic acid sequences in a clinical specimen in a patient with radiologically documented lesion constitutes WHO definition of a confirmed case (Brunetti *et al.* 2010). However, false negative PCR may occur because alveolar cysts are heterogeneous and biopsy material may not include sufficient amounts of cestode DNA (Bulakcici *et al.* 2016; Hemphill *et al.* 2019). Furthermore, puncture biopsies may be associated with a risk of metastatic spread due to disruption of parasitic membranes (Hemphill *et al.* 2019).

Treatment of AE generally involves a combination of surgery and long-term antihelminthic therapy. In many cases radical resection of the entire infected portion of the liver is effective, but the size or location of the lesion and presence of metastasis can render the disease inoperable (Mihmanli *et al.* 2016; Hemphill *et al.* 2019). Benzimidazoles may be required for years to life to prevent disease progression or recurrence, particularly when surgery is not an option, and can increase 15-yr survival from ~0% to 50–80% (Mihmanli *et al.* 2016; Hemphill *et al.* 2019).

Transmission and Prevention

Rates of human infection tend to be significantly lower than the rates of human exposure, suggesting *E. multilocularis* has low transmissibility to humans and infection generally requires repeated long-term exposure (reviewed in Vuitton 2003; Provlab Alberta 2018). The transmission of *E. multilocularis* from definitive to intermediate hosts is fecal-oral, and human infection, therefore, depends on consumption of eggs (CDC 2019).

Indirect contact with animal feces contaminating soil (parks, playgrounds, property land), plants (garden vegetables, berries), or water is a major risk factor. This is particularly significant in Canada with urban coyotes entering parks and backyards and contaminating the environment with their feces. Companion and working dogs could become infected through ingestion of intermediate

rodent hosts and thus become a source of infection to humans through direct contact as eggs may linger on the fur of infected animals. The same applies to handling furs or carcasses of other canids such as coyotes by hunters or trappers, particularly if PPE (personal protective equipment) is not used and hand hygiene is not practiced. Some regional or cultural behaviours, such as running kitchen gardens, growing organic foods, gathering wild plants, hunting, trapping, hiking, and agricultural work all constitute potential risk factors (Kern *et al.* 2003).

There are no universally approved recommendations for disease prevention at this stage, but thorough washing or cooking vegetables and fruit prior to ingestion, regular deworming of pets (particularly those exposed to rodents such as in off-leash areas) and strict hand hygiene is currently the most practical approach to reduce the risk of infection. Hand hygiene and avoidance of aerosol generating procedures when handling infected, or potentially infected, material (or use of safety cabinets) as well as use of PPE when necessary is most appropriate in a workplace setting. Given high rates of infection among wild canids in Canada, furs, feces, and tissues of these animals, alive or dead, should always be handled with care.

CONCLUSION

Mutant strains of invasive *E. multilocularis* are widespread and common in Canada. These may be a greater risk to human health than native strains, and because of their pathogenicity, it is recommended anyone who handles canids or could indirectly come into contact with their feces take extra precautions.

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