Alveolar Echinococcosis (E. multilocularis)

Human alveolar echinococcosis (AE) is a potentially lethal infection caused by Echinococcus multilocularis, an intestinal tapeworm of carnivorous animals such as foxes, coyotes and domestic dogs. Humans are infected by ingestion of eggs excreted in animal feces, directly or through contaminated soil; as accidental hosts they harbour a larval form of the disease and therefore are not able to transmit infections to other humans or animals. See parasite life cycle for more information.

E. multilocularis is a parasite adapted to cold/arctic climates; its eggs will survive in fall/winter for appx 1 year (temperature range of -30 to +60^{O}C)

AE presents in humans as a continuously growing tumour-like polycystic mass, involving the liver in 99% of cases, with the potential for local or metastatic spread to distant organs. Incubation period is estimated at 5–15 years; mortality rate is >90% within 10-15 years of diagnosis (if untreated, or inadequately treated). The disease is more severe and progresses more rapidly in immunocompromised patients

Relatively high prevalence (up to 60%) of *E. multilocularis* s was recently found in urban coyotes in Calgary and Edmonton; presence of the parasite was also noted in Alberta dogs. While only one case of human AE has previously been described in Canada (Manitoba, 1928), a cluster of ten cases was reported in Alberta between 2013 and 2018. This represents a significant increase over a short period of time and underlines a risk for emergence of this disease in the province, continuing into the future.

Presence of the pathogen in urban coyotes and dogs and its transmission to humans emphasizes the importance of this disease, and the need for diagnostic awareness and appropriate countermeasures

Laboratory diagnosis of AE in human relies mainly on findings by imaging techniques, supported by immunodiagnostic tests, in a relevant epidemiological/clinical setting (symptoms and signs may vary with location, size and type of the lesion). Definitive diagnosis is obtained by histopathology and or detection of *E. multilocularis* nucleic acid sequence(s) in a clinical specimens (tissue biopsies).

Imaging

Ultrasonography is the basis of AE diagnosis at individual and population levels, and should be used as first-line imaging technique. Typically, findings would show pseudoneoplastic intrahepatic mass with a hyper and hypoechoic areas with scattered calcifications and irregular, poorly defined edges, or pseudocyst – like area of central necrosis irregular hyperechogenic ring. Nodular or scattered calcified lesions in the liver are not uncommon and, if accompanied by positive serology, may represent abortive infection
CT allows anatomical and morphological characterization of AE lesions, and is particularly useful for extensively calcified lesions and preoperative evaluation. MRI may reveal characteristic multivesicular morphology, thus supporting the diagnosis in unclear cases.
See sample diagnostic images.

Immunodiagnostics

Serology usually supports diagnosis already suspected by ultrasonography/other imaging. Currently available immunodiagnostic tests allow discrimination between alveolar and cystic echinococcosis (caused by *Echinococcus granulosus*). The use of purified/recombinant antigens (such as Em2, Em18, EmVF) has a high diagnostic sensitivity, and specificity, approaching 100% when tested in combination (ELISA screen plus confirmatory Western Blot). Serology (Em18) may be used to assess viability of the parasite as well.

Direct assessment: **histopathology**

The diagnosis of AE can be confirmed by the identification of necrotic irregular cystic cavities most often without fibrous rimming. The cysts are lined by a laminated membrane and most often do not contain nucleated germinal membranes or protoscolices that can be seen in no more than 15% of cases. The laminated membranes are often fragmented and can be highlighted by strong positive expression of Periodic Acid Schiff (PAS) or Gomori Methenamine Silver (GMS) stains. These structures invade necrotic liver tissue eliciting a variable host response with occasional granulomatous reaction including neutrophils, eosinophils and lymphocytes or peripheral rim of fibrosis and focal calcification. See selected pathology images.

Direct assessment: **PCR**

Microscopic diagnosis of fine-needle biopsy (FNB) specimens or old calcified samples may be difficult, so PCR targeting the E. multilocularis-specific nucleic acids in tissue/fine needle aspiration biopsies can be used to confirm diagnosis. RT PCR may be used to assess viability of the parasite as well. However, a negative PCR on FNB does not rule out the disease and negative RT PCR is not indicative of complete inactivity of the lesion.
NOTE: any puncture/ needle biopsy of the liver may carry a risk of dissemination and thus requires post-interventional chemotherapy.

All serology, PCR and RT-PCR is currently referred to an external reference lab. *Consultation with Microbiologist on call is required prior to specimen collection/submission. At least three days' notice is required for PCR requests.*

Role of serology in diagnosis of alveolar echinococcosis

Positive serology in exposed individuals may be seen in following four situations 1) "patent", overt, symptomatic disease, 2) "latent", non-apparent disease, 3) presence of fully calcified late "abortive" lesions representing dead parasite and 4) early abortive infection with no detectable lesions, representing resistance.

Since the time interval between infection and disease is anywhere from 5-10 years, and time from ingestion of eggs to seroconversion is unknown, **a single negative serology result does not rule out infection and a single positive result does not rule in presence of the disease.** This makes serology alone (without imaging) of doubtful value as a diagnostic or screening tool in

epidemiologically or clinically suspected alveolar echinococcosis (of note - the largest mass-screening program carried out in Hokkaido, Japan showed prevalence of asymptomatic AE to be 0.008% of the total)

For these reasons, any serology requests for suspected AE cases have to fulfill the following criteria:

Adequate exposure: humans are unsuitable hosts for E. multilocularis , therefore animal contact has to be considerable in terms of time and intensity (repeated or long term close exposure, as opposed to casual contact or travel to endemic areas)

Proper evidence of infection in the source animal such as E. multilocularis, namely, PCR of dog feces or eggs obtained from dog feces. Several commercial veterinary kits are available for this purpose. Microscopic detection of eggs in animal feces is not sufficient for diagnosis of AE, as Taenia or Echinococcus spp eggs are morphologically indistinguishable from each other. Canids diagnosed
with visceral involvement (larval disease) have to be additionally tested for adult worm disease/eggs excretion before being considered as posing a risk to humans

Presence of ultrasound documented characteristic liver lesions.
Note – for suspect cases, ultrasound screening should be carried out no earlier than 12 months (6 months in immunocompromised patients) after documented exposure. (D. Vuitton, WHO Collaborating Centre, Université Bourgogne Franche-Comté, personal communication)

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