Alveolar echinococcosis: what triggers emergence in North America, Central Europe and Asia?

Bruno Gottstein\textsuperscript{a} and Peter Deplazes\textsuperscript{b}

Purpose of review
Infection with the larval (metacestode) stage of \textit{Echinococcus multilocularis} causes alveolar echinococcosis (AE), a serious hepatic disorder. The parasite has increased its infection extensity in wildlife and domestic dogs, mainly due to urbanization and spatial extension of wildlife hosts in Europe, Asia as well as North America, resulting in emerging infection risk for humans.

Recent findings
In hyperendemic areas such as Kyrgyzstan and China, ecological and socioeconomic changes have been associated with the unpredictable increase of AE cases. In North America, the appearance of the European-like genotype is of concern. In Europe, the annual increase of human case numbers reached a plateau even in hyperendemic situations. Therefore, we conclude that most of the exposed individuals are resistant to parasite invasion and/or to disease development. Thus, AE develops in a few healthy individuals, but preferentially in immunosuppressed patients.

Summary
In the future, improved diagnostic strategies will allow more precise estimations of transmission routes including the role of food, water and direct dog contact, which should yield improved public health recommendations. Finally, understanding protective innate and acquired immune mechanisms as well as parasite-driven immune-evasion processes will be essential to develop curative therapies in nonoperable patients and, futuristically, appropriate vaccines.

Keywords
endemicity, immunity, infection extensity, populations, resistance, susceptibility

INTRODUCTION
The cestode parasite \textit{Echinococcus multilocularis} circulates between wild and domestic canids as definitive, and rodent species as intermediate hosts. Infectious eggs are fecally released into the environment by the definitive hosts. Parasite eggs can persist for several months or even longer. Once such eggs (containing a fully embryonated larvae - the oncosphere) have been orally ingested by an intermediate host, the larval stage (metacestode) will primarily develop in the liver. Asexual reproduction upon formation of high numbers of protoscoleces occurs. These will develop into adult worms after predation by a definitive host. Eggs may also develop into the metacestode stage in various mammalian species (dead-end hosts, e.g. pigs, primates, dogs and, importantly, also humans). The metacestode infection, when progressing, results in alveolar echinococcosis (AE) – a cancer-like disorder primarily affecting the liver, with a subsequent potential to secondarily spread into adjacent organs or even to remote sites (e.g. brain). If not appropriately treated, AE will lead to a lethal outcome [1].

Due to an increasing intertwining of the above-mentioned canid-vole life cycle with human settlements and civilization, including urban zones, humans are increasingly exposed to this zoonotic parasite. This phenomenon has been mainly driven both by an urbanization event of foxes and coyotes (thus spreading parasite eggs within close vicinity of human residences, establishing an urban cycle of the parasite with voles), and by the fact that...
domestic dogs with free access to voles are playing an important role in exposing humans to parasite eggs by close physical contact to their owners, and also by contributing to the environmental (fecal) contamination with parasite eggs in human habitation zones [2].

Geographically, *E. multilocularis* and thus AE are confined to the northern hemisphere, specifically to northern and central regions of Asia, Central Europe and parts of North America [3]. In the last decade, studies or reviews focusing on these ecologically very distinct and geographically not overlapping endemic areas, claiming ‘emergence of AE’ have been published, e.g., globally [3], North America [4], Europe [5], Asia [6**,7] and Kyrgyzstan [7,8]. However, it is important to mention that the number of cases and the burden of AE is unequely distributed in these endemic areas, with approximately <0.5% of AE cases in North America, < 5% in Europe and accordingly approximately 95% in Northern and especially Central Asia, with China documenting the majority of the burden [3].

**INCREASE OF AE CASE NUMBERS AND EXPANSION IN DIFFERENT ENDEMIC AREAS**

**Europe**

Presently, a rough estimate of approximately 200–300 new AE cases per year is expected to occur all over Europe. The annual average AE incidence in Europe is relatively low with 0.03 to <1 cases per 100,000 inhabitants, with few endemic spots reaching slightly higher incidences. In Europe, AE has long been diagnosed in a ‘historical’ focus, including eastern parts of France, southern Germany, Switzerland, and western Austria [5]). Within the past decades, however, increasing numbers of human AE cases have been documented in many other European areas where the disease had not been recognized before, especially in the Baltic area and Poland [9]). Interestingly, as compared to the expansion of the endemic area to the West, North and East of Europe [5], the respective southern border of the endemic area has not expanded much to the South. A stable situation regarding *E. multilocularis* endemicity in the south of the Alps in Switzerland was associated with the overlapping occurrence of the vole species *Microtus arvalis*, either absent or only scarcely prevalent in this southern zone [10], indicating that parasite transmission in a given area is mostly determined by the presence of not only definitive hosts, but as importantly by appropriate intermediate hosts. In the southern endemic border of *E. multilocularis*, new cases were reported in restricted areas from the southwestern Italian Alps close to the Austrian border and more recently in the southwestern Italian Alps, near the French border [11]. In Croatia [12], a high endemicity of *E. multilocularis* was shown in foxes, and later the first record of a human AE case was documented [13], showing the importance of documenting the occurrence of this zoonosis especially on the border of any endemic area.

In Europe, for the past decades, the increasing transmission of *E. multilocularis* was associated with the increase of fox populations after an efficient rabies control. Romig et al. [14] estimated that the infection pressure with *E. multilocularis* eggs increased in the German hyperendemic area by a factor of 10. A significant increase in AE incidences was documented for Switzerland around 10 years after the rise of the fox population, highly affected by *E. multilocularis* [15]. Increases of case numbers have also been documented in Lithuania, Poland and adjacent European areas [9,16*]. Even though case registration is not perfect and underreporting has to be considered, the increase appears to stabilize more or less at a higher, but still relatively low level in most of these European zones. Thus, the increase of environmental *E. multilocularis* egg contamination, leading to a much higher exposure risk for humans especially in urban areas, should theoretically cause higher annual incidences of AE. Conclusively, a better understanding of the mechanisms contributing to the ‘proposed’ relative high resistance of humans exposed to the parasite is needed, and is thus addressed and discussed in the following sections Q2 and Q3.

**North America**

*E. multilocularis* was historically known to occur in the very scarcely populated Northern Tundra Zone, including the northwestern coastal region of Alaska.
and the Western Canadian Arctic, and also the Northern Central Region, including the Canadian provinces Alberta, Saskatchewan and Manitoba and some northcentral states of the USA [17]. The *E. multilocularis* genotypes in these endemic areas belong either to the N1 or the N2 group, as revealed by later molecular analyses [17,18], and a relatively low number of humans cases, except in the northwestern coastal regions of Alaska (e.g. St. Lawrence Island) was found. Since 2009, however, hepatic AE has been documented in several dogs [19]. Such cases as a first epidemiological hint for emergence is unusual, as it probably reveals the tip of an iceberg reflecting an overall high endemic situation in wildlife (as known since the eighties for Central Europe). Following the first canine AE case in British Columbia [20], further cases were recorded in Alberta, Saskatchewan, Manitoba and Ontario [21]. Subsequent epidemiological investigations in wildlife, such as in coyotes, foxes and also rodent hosts, confirmed the presence of this parasite in western Canada (reviewed in [22]).

Molecular analyses revealed that these emerging *E. multilocularis* isolates belong to the European genotype assemblage, and are distant from the two manifest American genotypes N1 and N2 [17,23]. In 2013, a human AE case was diagnosed at the Alberta Provincial Laboratory for Public Health, and since 2016, more human AE cases caused by isolates of the European assemblage were detected in Canada [23,24]. More recently, this ‘European’-like genotype was newly found in the United States upon an intestinal infection in a dog from Missouri [25], and the first human isolate belonging to the same assemblage east of the Mississippi River was just published by [26].

This new epidemiological situation in North America is best explained by the recent importation of dogs or red foxes from Europe [4] and in the future more cases can be expected, perhaps reaching a similar disease burden as in Europe, especially in densely populated areas.

**North and Central Asia**

*E. multilocularis* is widespread in northern Asia with around 90% of human AE cases documented in China, but increase of AE cases has also been documented in southern Siberia (e.g. Altai, Omsk and Tomsk, central Yakutia and parts of the Far East). The history of AE in China was well-reviewed by [27], including high endemic areas in relatively sparsely populated regions of the inner Mongolia, the north-western of China (e.g. Gansu, Qinghai, Ningxia, Xinjiang) and the Southwestern and Central China (Sichuan and the Tibet Autonomous Region). Parasite transmission is characterized by a high variety of definitive and intermediate hosts with major key species contributing to the cycle [28]. Therefore, this complex ecology will not be the focus of this opinion paper. Furthermore, the literature documenting environmental factors related to the transmission of *E. multilocularis* in China and presented references dealing with social risk factors identified for AE were recently reviewed [6**]. Not only in China, but all over the Asian endemic AE area, stray and owned domestic dogs seem to play a central role in the transmission of *E. multilocularis* eggs within human settlements. Thus, the primary parasite cycle appears to be maintained in most of the areas in wildlife, but the spill-over to humans is considered to occur predominantly with domestic dogs infected in pastures surrounding rural settlements [29].

Recent findings from Kyrgyzstan evidenced an extraordinary high annual AE surgical incidence of 3.02 cases per 100,000 inhabitants at the overall country-level level, but up to 246 cases per 100,000 at local community levels [8]. Similarly to many areas in China, the infection pressure in these rural communities can be considered as very high based on critical hygienic conditions, unsafe water supply and high wildlife, and especially domestic dog prevalence of the parasite [6**,30]. Certainly, the respective epidemiological situation significantly differs from that of Europe. Although the prevalence in foxes may be comparable in both areas, domestic dogs are much less affected in Central Europe, as these have most likely lower access to intermediate hosts and are more frequently dewormed. Furthermore, in both of these Asian areas, genetic analyses of various parasite isolates revealed that most mitochondrial genotypes belong to the Asian assemblage [6**,31]. Interestingly, for Kyrgyzstan, the medium age of patients at the time of diagnosis of AE was much lower than in Europe [1,7,8] or even China [6**,32], indicating different dynamics in disease development. However, the overall age distribution may considerably differ between these areas, and this has to be taken into consideration in future respective studies. Consequently, the around 100 times higher incidences of AE in Kyrgyzstan as compared to Europe cannot convincingly be explained with the differences in infection pressure only. More research is needed to elucidate the role of immunogenetics of different human populations, parasites’ genetics and other so far neglected research areas such as (i) human host intestinal micro- and macrobiome, (ii) co-infection with other organisms, such as intestinal nematodes especially in children, and (iii) nutritional status and eating habits, among others.
The above outlined diverse epidemiological situations raise the following questions:

**Q1: SHOULD E. MULTILOCULARIS BE CONSIDERED AS A ZOONOTIC GEOHELMINTH, E.G. FOOD AND A WATER BORNE PARASITE?**

Due to the long (asymptomatic) incubation time till disease (approximately 5–15 years), it is relatively difficult to elucidate and thus understand transmission pathways and associated infection risk factors in humans [33,34]. In general, plenty of environmentally resistant helminths and protozoal stages are transmitted to humans by oral uptake. For *E. multilocularis*, depending on the endemic areas, free-roaming domestic dogs and wild canids (e.g., foxes, coyotes) play a major role in contaminating the environment, including recreational areas, private kitchen gardens and agricultural areas with infectious eggs. Increasing populations of red foxes in Europe, and coyotes in Northern America have raised concerns about changing infection dynamics in endemic areas. However, the transmission of *E. multilocularis* eggs to humans is still not well understood in the very diverse epidemiological situations, but it can essentially be attributed to contaminated food, water, environment and human behavior, especially the hand-to-mouth contact [35]. For example, dog ownership has been identified as a risk factor for patients with AE in Europe [33,36], Kyrgyzstan [7] and China [6].

Convincing quantitative estimations of the role of food and other ways of transmission are not yet known in order to yield specific prevention measures. New diagnostic strategies are focusing on the detection of orally transmitted pathogens, including *E. multilocularis*, in the frame of the whole food production chain [35]. A methodology with the potential to simultaneously detect a defined range of environmentally persistent parasite stages of possible foodborne pathogens has recently detected *E. multilocularis* eggs in lettuce sold on the market for human consumption in Switzerland [37]. However, we would like to stress the fact that amplification of *E. multilocularis* DNA does not imply that infectious eggs are present. Beside methodical concerns that have been critically discussed [31], DNA can originate from ‘mummified’ eggs. For *E. multilocularis* eggs, it is possible to assess their viability for experimental studies with an in vivo method using subcutaneous injection in mice but this method was considered not to be sufficiently sensitive and practical for environmental samples [37].

Although there is – from the epidemiological point of view – evidence of possible food, water and hand-to-mouth transmission with *E. multilocularis* eggs, we still lack the detailed information needed about the effective infection sources in various population/patient groups, which would allow to elaborate solid, effective and efficient prevention measures that could be recommended for all individuals living in different lifestyles in different cultural settings and hygienic conditions.

**Q2: IS AE AN ‘OPPORTUNISTIC’ DISEASE?**

There is accumulating evidence that the immune fitness and respective response upon *E. multilocularis* infection are crucially involved in the host-parasite interplay. A weak or impaired immune responsiveness definitively promotes metacestode proliferation and metastatic progression, such as following liver transplantation, or rarely upon immune deficiency by Acquired Immune Deficiency Syndrome (AIDS) (reviewed in [38]) However, it is important to state that during the AIDS epidemics, only very few AE cases have been documented, and AE has not been ranked as important AIDS-associated opportunistic disease. Therefore, we conclude that susceptibility to AE development in AIDS patients is based on a compromised acquired immunity most likely after establishment of the infection, and not on the first level of resistance toward parasite invasion, where e.g. (and presumably) innate immune mechanisms are involved (see below). This hypothesis is supported by the observation that inoculation of *E. multilocularis* eggs in nude rats did not result in AE [39]. AE has also been increasingly reported in patients receiving immunosuppressive therapy for malignant and inflammatory diseases or after a transplant [4,40–42]. A recent Swiss study reported a fourfold increase of AE cases in immunocompetent patients, and a tenfold increase in immunocompromised patients in the same time span [43]. Interestingly, analyzing the kind of immuno-impairment revealed that immunosuppressive conditions included malignancies (36%), auto-immune diseases or immunosuppressive therapies (31%), concomitant infectious diseases (11%), chronic asthma conditions (9%), previous transplantations (4%) and other not further specified immunocompromising conditions (9%).

So far, only a few epidemiological studies have indicated that probably most human individuals exposed to infection with *E. multilocularis* eggs do not develop AE. This hypothesis was either based on investigating seroconversion rates in exposed populations with seropositives not developing AE for a long time period, or seropositives presenting fully calcified intrahepatic *E. multilocularis* lesions, confirmed by histology and/or polymerase chain
reaction [44–47]. In a previous speculative appraisal, we estimated an approximate ratio of 1 clinically developing AE case per 100 individuals exposed to infection (based on serology), i.e., the largest part of ‘infected’ individuals will thus present resistance to disease [5].

Finally, there is evidence that the increasing administration of immunomodulatory treatments in humans of predominantly industrialized AE-endemic areas has resulted in a shift in the ratio between immunocompetent toward immunosuppressed AE-patients. In France and in Switzerland, approximately 20% of the newly diagnosed AE patients experienced a previous immunotherapy in their medical report, whereas this was below 2% 20 years ago (S. Bresson-Hadni and G. Beldi, personal communication). As the infection time point could not retrospectively be determined in these studies, more prospective precise studies are needed to elucidate if AE should be considered as an opportunistic infection.

Q3: WHAT IS THE BASIS OF ‘RESISTANCE’ OR DISEASE (ALVEOLAR ECHINOCOCCOSIS) DEVELOPMENT?

We know that the metacestode of *E. multilocularis*, once established in the liver, outwardly protects with a tight layer represented by a carbohydrate-rich extracellular matrix, termed the laminated layer (LL). Biochemical analyses of this LL showed that the non-decorated cores of the matrix, together with Galp\(1-3\)(Galp\(1-4\)Galp\(1-4\)GlcNAcp\(1-6\))GalNAc, comprise over 96% of the glycans in molar terms [[48]]. This LL is crucial for parasite survival and proliferation as it is able to protect the parasite from host’s innate or subsequent specific immune reactions [49]. Therefore, all previous investigations successfully using vaccines against *E. multilocularis* were based on an immune response targeting the oncosphere before LL development [50,51]. Once the infection leads to early stage AE, we know that the variable clinical forms of disease development in individuals are dependent upon immunological fitness and orientation [38**]. However, little is known why probably a major part of the exposed individuals does not develop AE. So far, only descriptive documentations of single ‘abortive’ or ‘died-out’ AE cases have been published and no systematic study has been performed to address immunological parameters in a defined cohort of AE resistant individuals, neither in those demonstrating the presence of fully calcified AE lesions, nor in individuals presenting a specific AE-positive serology without hepatic lesion formation. The remaining key questions to address focus on (i) the role of immunogenetics, (ii) the role of innate immune mechanisms interfering with oncosphere invasion and early development and (iii) the role of acquired immunity.

**Immunogenetics**

Few studies so far indicated that genetic variation of the human leukocyte antigen (HLA) system may be associated with the occurrence and/or progression of AE lesions in humans, and patients with the HLA-DR3 DQ2 haplotype were shown to have more severe disease (reviewed in [38**]). However, no data have yet been published regarding any clear immunogenetic predisposition for resistance after infection, or about immunogenetic markers that may account for becoming diseased.

**Innate immunity**

Information concerning the immunological mechanisms, which may be associated with resistance, can be found in experimental murine/rodent studies. Susceptibility to *E. multilocularis* infection considerably varies among intermediate (mostly rodents) and dead-end host species (e.g. humans and pig), in particular regarding intestinal oncosphere invasion and subsequent hepatic metacestode development. A recent study [52] showed that Wistar rats are highly resistant to oncosphere invasion after eggs inoculation. However, after immunosuppressive treatment with dexamethasone, rats become susceptible. The key element found were granulocytes (polymorphonuclear cells, PMN), as the majority of PMN-depleted animals developed liver metacestodes within 10 weeks after infection [53*]. With regard to full resistance that protects from disease, pigs were shown to be appropriate for studying resistance mechanisms. Already under natural infection conditions, pigs get infected, but do not develop disease, as the forming lesions die-out and calcify [54]. This phenomenon occurs under experimental infection within a month after egg inoculation [55]. However, this model has not yet been used to study in detail the immune parameters responsible for AE resistance. First approaches in this direction were recently published by [56], who identified various innate parameters as putative key-players in the host-parasite cross-talk.

**Acquired immunity**

Most experimental murine studies so far addressed the role of various cellular or cytokine components by using correspondingly immunodeficient animals, from which a causative association between a missing immune parameter and increased disease development can be concluded. A respective summary is
provided in Table 1 (refs to Table 1: [57–64] which will herewith not be further discussed in detail. Synoptically, all of these studies evidenced that a periparasitically induced immune anergy is one of the key mechanisms of the metacestode immune evasion which represents a strong interconnection with regulatory T-cell mechanisms. The logical conclusion would then be that AE-resistant individuals developed mechanisms neutralizing or abrogating parasite immune evasion which occurs most likely via immunoreactive parasite metabolites [65] or other metacestode metabolites [66]. Indeed, a recent study [67] documented parasite-derived antigenic particles (spems) by immunohistochemistry to contain Em2-epitopes and to have immunomodulating properties. These findings further document systemic and peri-parasitic immunological events associated with AE. Deeper understanding of these immunomodulating mechanisms will support the development of immunotherapeutics to treat AE and/or to curatively support conventional parasitostatic medication with benzimidazoles.

As with other taeniid cestodes, immunization of intermediate hosts with *E. multilocularis* recombinant oncospheral antigens have demonstrated efficacy by preventing disease development in experimentally infected susceptible rodents [50,51,68] and first vaccination trials have now been carried out in primates [69].

**CONCLUSION**

Besides important research in epidemiology, diagnosis and clinical management of AE, source attribution with *E. multilocularis* eggs, factors determining parasite invasion in the first phase of infection as well as mechanisms of resistance limiting the parasite invasion or disease development have still to be studied in depths; the following key points include then:

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**Conflicts of interest**

There are no conflicts of interest.

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**REFERENCES AND RECOMMENDED READING**

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest


Review excellently highlighting the recent research findings acquired in the globally main endemic region for *E. multilocularis*.


Tropical and travel-associated diseases


Excellently presented problem analysis of E. multilocularis in Canada.


Excellently presented problem analysis of E. multilocularis in the USA.


Review excellently highlighting the recent research findings on E. multilocularis globally.


Review excellently highlighting the recent research findings on E. multilocularis globally.


