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

Bruno Gottstein[a] *and Peter Deplazes*[b]

Purpose of review
Infection with the larval (metacestode) stage of *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 *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 residencies, establishing an urban cycle of the parasite with voles), and by the fact that

[a]Institute of Infectious Diseases, Faculty of Medicine, University of Bern, Bern and [b]Institute of Parasitology, Vetsuisse Faculty and Faculty of Medicine, University of Zurich, Zurich, Switzerland

Correspondence to Bruno Gottstein, 1Institute of Infectious Diseases, Faculty of Medicine, University of Bern, Friedbühlstrasse 51, CH-3001 Bern, Switzerland. Tel: +41 31 664 06 27;
e-mail: bruno.gottstein@ifik.unibe.ch

Curr Opin Infect Dis 2021, 34:440–446
DOI:10.1097/QCO.0000000000000765

KEY POINTS

- Main risks of infection still not elucidated and not quantified.
- Experimentally, factors of innate immunity are essential to trigger oncosphere penetration into the intestinal mucosa and subsequent intrahepatic establishment.
- Predisposition to develop human AE in immunocompetent individuals is unknown.
- Acquired immunity, if not running into anergy, may induce immunoprotection and can thus open new approaches for immunotherapy or vaccination.

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▪▪] and Kyrgyzstan [7,8]. However, it is important to mention that the number of cases and the burden of AE is unevenly 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 spillover 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 Galpβ1-3(Galpα1-4Galpβ1-4GlcNAcpβ1-6)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

Table 1. Outcome of immunomodulation in experimental murine AE

Immunological parameter studied	Resistance increased	Refs.
FoxP3+ Tregs depletion	Yes	[57]
TGFβ-blockage	Yes	[57,58]
iNOS-blockage	Yes	[59]
IFNα-2α, IFNγ, Il-12, TNFα increased	Yes	Reviewed in [60]
FGL2 deficiency	Yes	[61]
PD1/PDL1-blockage	Yes	[62,63]
TIGIT-blockage	Yes	[64]

FGL2, Fibrinogen-like protein 2; iNOS, Inducible Nitric Oxide Synthase; IFNγ, Interferon gamma; PD1/PDL1, Programmed cell death protein 1 / Programmed cell death 1 ligand 1; TGFβ, Transforming Growth Factor beta; TNFα, Tumor Necrosis Factor alpha; TIGIT, T cell immunoreceptor with Ig and ITIM domains.

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 peri-parasitically induced immune anergy is one of the key mechanism 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 immunoactive parasite metabolites [65] or other metacestode metabolites [66]. Indeed, a recent study [67] documented parasite-derived antigenetic 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:

Acknowledgements

None.

Financial support and sponsorship

No funding has been received for this work.

Conflicts of interest

There are no conflicts of interest.

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

1. Kern P, Menezes da Silva A, Akhan O, *et al.* The echinococcoses: diagnosis, clinical management and burden of disease. Adv Parasitol 2017; 96:259–369.
2. Liccioli S, Giraudoux P, Deplazes P, Massolo A. Wilderness in the 'city' revisited: different urbes shape transmission of *Echinococcus multilocularis* by altering predator and prey communities. Trends Parasitol 2015; 31:297–305.
3. Deplazes P, Rinaldi L, Alvarez Rojas CA, *et al.* Global distribution of alveolar and cystic *echinococcosis*. Adv Parasitol 2017; 95:315–493.
4. Massolo A, Liccioli S, Budke C, Klein C. *Echinococcus multilocularis* in North America: the great unknown. Parasite 2014; 21:73.
5. Gottstein B, Stojkovic M, Vuitton DA, *et al.* Threat of alveolar echinococcosis to public health – a challenge for Europe. Trends Parasitol 2015; 31:407–412.
6. ■■ Fu MH, Wang X, Han S, *et al.* Advances in research on echinococcoses epidemiology in China. Acta Trop 2021; 219:105921.

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

7. Bebezov B, Mamashev N, Umetaliev T, *et al.* Intense focus of alveolar echinococcosis, South Kyrgyzstan. Emerging Infect Dis 2018; 24:1119–1122.
8. Paternoster G, Boo G, Wang C, *et al.* Epidemic cystic and alveolar echinococcosis in Kyrgyzstan: an analysis of national surveillance data. Lancet Glob Health 2020; 8:e603–e611.
9. Marcinkut≒ A, Šarkūnas M, Moks E, *et al. Echinococcus* infections in the Baltic region. Vet Parasitol 2015; 213:121–131.
10. Guerra D, Hegglin D, Bacciarini L, *et al.* Stability of the southern European border of *Echinococcus multilocularis* in the Alps: evidence that *Microtus arvalis* is a limiting factor. Parasitol 2014; 16:1–10.
11. Critterio CV, Obber F, Trevisol K, *et al.* Echinococcus multilocularis and other cestodes in red foxes (Vulpes ulpes) of northeast Italy, 2012-2018. Parasites Vectors 2021; 14:29.
12. Beck R, Mihaljević Ž. Brezak R, *et al.* First detection of *Echinococcus multilocularis* in Croatia. Parasitol Res 2018; 117:617–621.
13. Dušek D, Vince A, Kurelac I, *et al.* Human Aaveolar echinococcosis, Croatia. Emerg Infect Dis 2020; 26:364–366.
14. Romig T, Thoma D, Weible AK. *Echinococcus multilocularis*–a zoonosis of anthropogenic environments? J Helminthol 2006; 80:207–212.
15. Schweiger A, Ammann A, Candinas D, *et al.* Human alveolar echinococcosis after fox population increase, Switzerland. Emerg Inf Dis 2007; 13:878–882.
16. ■ Fischer I, Graeter T, Kratzer W, *et al.* Distribution of alveolar echinococcosis according to environmental and geographical factors in Germany, 1992-2018. Acta Trop 2020; 212:105654.

Review excellently highlighting the recent research findings on *E. multilocularis* in Germany.

17. Gesy KM, Jenkins EJ. Introduced and native haplotypes of *Echinococcus multilocularis* in Wildlife in Saskatchewan. Canada. J Wildl Dis 2015; 51:743–748.
18. Spotin A, Boufana B, Ahmadpour E, *et al.* Assessment of the global pattern of genetic diversity in *Echinococcus multilocularis* inferred by mitochondrial DNA sequences. Vet Parasitol 2018; 262:30–41.
19. Jenkins EJ, Peregrine AS, Hill JE, *et al.* Detection of European strain of *Echinococcus multilocularis* in North America. Emerg Infect Dis 2012; 18:1010–1012.
20. Peregrine AS. Alveolar echinococcosis in dogs: an emerging issue? Vet Rec 2015; 177:567–568.

21. Kotwa JD, Isaksson M, Jardine CM, *et al. Echinococcus multilocularis* infection, Southern Ontario, Canada. Emerg Infect Dis 2019; 25:265–272.

22. ■ Houston S, Belga S, Buttenschoen K, *et al.* Epidemiological and clinical characteristics of alveolar echinococcosis: an emerging infectious disease in Alberta, Canada. Am J Trop Med Hyg 2021; 104:1863–1869.
Excellently presented problem analysis of *E. multilocularis* in Canada.

23. Massolo A, Klein C, Kowalewska-Grochowska K, *et al.* European *Echinococcus multilocularis* identified in patients in Canada. NEJM 2019; 381:384–385.

24. Trotz-Williams LA, Mercer NJ, Walters JM, *et al.* Public health follow-up of suspected exposure to *Echinococcus multilocularis* in Southwestern Ontario. Zoonoses Public Health 2017; 64:460–467.

25. Kuroki K, Morishima Y, Neil J, *et al.* Intestinal echinococcosis in a dog from Missouri. J Am Vet Med Assoc 2020; 256:1041–1046.

26. ■ Polish LB, Pritt B, Barth TFE, *et al.* First European Haplotype of *Echinococcus multilocularis* identified in the United States: an emerging disease? Clin Infect Dis 2021; 72:1117–1123.
Excellently presented problem analysis of *E. multilocularis* in the USA.

27. Vuitton DA, Wang Q, Zhou HX, *et al.* A historical view of alveolar echinococcosis, 160 years after the discovery of the first case in humans: part 1. What have we learnt on the distribution of the disease and on its parasitic agent? Chin Med J 2011; 124:2943–2953.

28. Romig T, Deplazes P, Jenkins D, *et al.* Ecology and life cycle patterns of *Echinococcus* species. Adv Parasitol 2017; 95:213–314.

29. Vaniscotte A, Raoul F, Poulle ML, *et al.* Role of dog behaviour and environmental fecal contamination in transmission of *Echinococcus multilocularis* in Tibetan communities. Parasitology 2011; 138:1316–1329.

30. Counotte MJ, Minbaeva G, Usubalieva J, *et al.* The Burden of Zoonoses in Kyrgyzstan: a systematic review. PLoS Negl Trop Dis 2016; 10:e0004831.

31. Alvarez Rojas CA, Kronenberg PA, Aitbaev S, *et al.* Genetic diversity of *Echinococcus multilocularis* and *Echinococcus granulosus* sensu lato in Kyrgyzstan: The A2 haplotype of *E. multilocularis* is the predominant variant infecting humans. PLoS Negl Trop Dis 2020; 14:e0008242.

32. Craig PS, Giraudoux P, Shi D, *et al.* An epidemiological and ecological study of human alveolar echinococcosis transmission in south Gansu, China. Acta Trop 2000; 77:167–177.

33. Conraths FJ, Probst C, Possenti A, *et al.* Potential risk factors associated with human alveolar echinococcosis: systematic review and meta-analysis. PLoS Negl Trop Dis 2017; 11:e0005801.

34. ■ Torgerson PR, Robertson LJ, Enemark HL, *et al.* Source attribution of human echinococcosis: a systematic review and meta-analysis. PLoS Negl Trop Dis 2020; 14:e0008382.
Review excellently highlighting the recent research findings on *E. multilocularis* globally.

35. Koutsunamis K, Allende A, Alvarez-Ordóñez A, *et al.* Public health risks associated with food-borne parasites. EFSA J 2018; 16:e05495.

36. Kern P, Ammon A, Kron M, *et al.* Risk factors for alveolar echinococcosis in humans. Emerg Infect Dis 2004; 10:2088–2093.

37. Guggisberg AR, Alvarez Rojas C, Kronenberg P, *et al.* A sensitive, one-way sequential sieving method to isolate helminths' eggs and protozoal oocysts from lettuce for genetic identification. Pathogens 2020; 9:624.

38. ■■ Wen H, Vuitton L, Tuxun T, *et al.* Echinococcosis: advances in the 21st Century. Clin Microbiol Rev 2019; 32:e00075–e00018.
Review excellently highlighting the recent research findings on E. multilocularis globally.

39. Armua-Fernandez MT, Joekel D, Schweiger A, *et al.* Successful intestinal *Echinococcus multilocularis* oncosphere invasion and subsequent hepatic metacestode establishment in resistant RccHan:WIST rats after pharmacological immunosuppression. Parasitology 2016; 143:1252–1260.

40. Kern P, Grüner B, Wahlers K. Diagnosis and course of echinococcocal diseases in the transplant setting. Transpl Infect Dis 2011; 13:217–221.

41. Chauchet A, Grenouillet F, Knapp J, *et al.* Increased incidence and characteristics of alveolar echinococcosis in patients with immunosuppression-associated conditions. Clin Infect Dis 2015; 59:1095–1104.

42. Dupont C, Grenouillet F, Mabrut JY, *et al.* Fast-growing alveolar echinococcosis following lung transplantation. Pathogens 2020; 9:756.

43. Lachenmayer A, Gebbers D, Gottstein B, *et al.* Elevated incidence of alveolar echinococcosis in immunocompromised patients. Food Waterborne Parasitol 2019; 16:e00060.

44. Rausch RL, Wilson JF, Schantz PM, McMahon BJ. Spontaneous death of *Echinococcus multilocularis*: cases diagnosed serologically (by Em2 ELISA) and clinical significance. Am J Trop Med Hyg 1987; 36:576–585.

45. Bresson-Hadni S, Laplante JJ, Lenys D, *et al.* Seroepidemiologic screening of *Echinococcus multilocularis* infection in a European area endemic for alveolar echinococcosis. Am J Trop Med Hyg 1994; 51:837–846.

46. Romig T, Kratzer W, Kimmig P, *et al.* An epidemiologic survey of human alveolar echinococcosis in southwestern Germany. Römerstein Study Group. Am J Trop Med Hyg 1999; 61:566–573.

47. Gottstein B, Saucy F, Deplazes P, *et al.* Is high prevalence of *Echinococcus multilocularis* in wild and domestic animals associated with disease incidence in humans? Emerg Infect Dis 2001; 7:408–412.

48. Wang J, von Gunten S, Beldi G, *et al.* Digest the Sugar, Kill the Parasite: a new experimental concept in treating alveolar echinococcosis. Pharmacology 2021; 106:3–8.

49. Gottstein B, Deplazes P, Aubert M. *Echinococcus multilocularis*: immunological study on the 'Em2-positive' laminated layer during in vitro and in vivo postoncospheral and larval development. Parasitol Res 1992; 78:291–297.

50. Siles-Lucas M, Merli M, Gottstein B. 14-3-3 proteins in *Echinococcus*: their role and potential as protective antigens. Exp Parasitol 2008; 119:516–523.

51. Gauci C, Merli M, Muller V, *et al.* Molecular cloning of a vaccine antigen against infection with the larval stage of *Echinococcus*. Infect Immun 2002; 70:3969–3972.

52. Joekel DE, Deplazes P. Optimized dexamethasone immunosuppression enables *Echinococcus multilocularis* liver establishment after oral egg inoculation in a rat model. Exp Parasitol 2017; 180:27–32.

53. ■ Joekel DE, Nur S, Monné Rodriguez J, *et al.* Agranulocytosis leads to intestinal Echinococcus multilocularis oncosphere invasion and hepatic metacestode development in naturally resistant Wistar rats. Parasitology 2021; 148:53–62.
Excellent research study on the role of innate immunity in experimental alveolar echinococcosis in a rat model.

54. Meyer A, Olias P, Schüpbach G, *et al.* Combined cross-sectional and case-control study on *Echinococcus multilocularis* infection in pigs in Switzerland. Vet Parasitol 2020; 4:100031.

55. Deplazes P, Grimm F, Sydler T, *et al.* Experimental alveolar echinococcosis in pigs, lesion development and serological follow up. Vet Parasitol 2005; 130:21–22.

56. Bakhtiar NM, Spotin A, Mahami-Oskouei M, *et al.* Recent advances on innate immune pathways related to host-parasite cross-talk in cystic and alveolar echinococcosis. Parasites Vectors 2020; 13:232.

57. Wang J, Müller S, Lin R, *et al.* Depletion of FoxP3(+) Tregs improves control of larval *Echinococcus multilocularis* infection by promoting co-stimulation and Th1/17 immunity. Immun Inflamm Dis 2017; 5:435–447.

58. Nono JK, Lutz MB, Breahm K. Expansion of host regulatory T cells by secreted products of the tapeworm *Echinococcus multilocularis*. Front Immunol 2020; 11:798.

59. Dai WJ, Waldvogel A, Jungi T, *et al.* Inducible nitric oxide synthase-deficiency in mice increases resistance to chronic infection *with Echinococcus multilocularis*. Immunology 2003; 10:238–244.

60. Wang J, Gottstein B. Immunoregulation in larval *Echinococcus multilocularis* infection. Parasite Immunol 2016; 38:182–192.

61. Wang J, Vuitton DA, Hemphill A, *et al.* Deletion of fibrinogen-like protein 2 (FGL-2), a novel CD4+ CD25+ Treg effector molecule, leads to improved control of *Echinococcus mutilocularis* infection. PLoS Negl Trop Dis 2015; 9:e0003755.

62. La X, Zhang F, Li Y, *et al.* Upregulation of PD-1 on CD4+CD25+ T cells is associated with immunosuppression in liver of mice infected with *Echinococcus multilocularis*. Int Immunopharmacol 2015; 26:357–366.

63. Wang J, Jebbawi F, Bellanger AP, *et al.* Immunotherapy of alveolar echinococcosis via PD-1/PD-L1 immune checkpoint blockade in mice. Parasite Immunol 2018; 40:e12596.

64. Zhang C, Lin R, Li Z, *et al.* Immune exhaustion of T cells in alveolar echinococcosis patients and its reversal by blocking checkpoint receptor TIGIT in a murine model. Hepatol 2020; 71:1297–1315.

65. Nono JK, Lutz MB, Brehm K. EmTIP, a T-Cell immunomodulatory protein secreted by the tapeworm *Echinococcus multilocularis* is important for early metacestode development. Negl Trop Dis 2014; 8:e2632.

66. Huang F, Dang Z, Zhang H, *et al.* Comparative study on secretome and transmembranome of immature and mature metacestodes of *Echinococcus multilocularis*. Vet Parasitol 2017; 245:153–159.

67. Grimm J, Nell J, Hillenbrand A, Henne-Bruns D, *et al.* Immunohistological detection of small particles of Echinococcus multilocularis and Echinococcus granulosus in lymph nodes is associated with enlarged lymph nodes in alveolar and cystic echinococcosis. PLoS Negl Trop Dis 2020; 14:e0008921.

68. Li R, Yang Q, Guo L, *et al.* Immunological features and efficacy of the recombinant subunit vaccine LTB-EMY162 against *Echinococcus multilocularis* metacestode. Appl Microbiol Biotechnol 2018; 102:2143–2154.

69. Lampe K, Gottstein B, Becker T, *et al.* Immunization of rhesus macaques with *Echinococcus multilocularis* recombinant 14-3-3 antigen leads to specific antibody response. Parasitol Res 2017; 116:435–439.