**Laboratory associated zoonotic parasitic infections: a review of main agents and biosecurity measures**

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**Abstract**

Laboratory workers are exposed to the risk of acquiring infections due to the manipulation of infectious materials. The biological hazard for researchers is seven times higher when compared with hospital and public health laboratory workers. Despite the implementation of standardized practices to control infections, multiple cases of Laboratory Associated Infections (LAIs) usually go unreported. There has been a lack of comprehensive epidemiological data regarding the situation of LAIs for parasitic zoonosis and besides, the available sources are not completely updated. Since most accounts of laboratory infections are organism-specific, this study has focused on common pathogenic/zoonotic species handled at parasitological laboratories and summarising the standard biosecurity protocols for the infectious agents. The main characteristics of *Cryptosporidium* spp., *Entamoeba* spp, *Giardia duodenalis*, *Toxoplasma gondii*, *Leishmania* spp., *Echinococcus* spp., *Schistosoma* spp., *Toxocara canis*, *Ancylostoma caninum*, *Strongyloides stercoralis* are considered in this review in order to assess the potential risk of developing occupational infections in the workplace along with stating prevention and prophylactic measures for each species. It was concluded that the LAIs from these agents can be prevented by using personal protective measures and good laboratory practices. However, further studies are necessary to better understand the environmental resistance of cysts, oocysts and eggs, with a view to select the most suitable disinfection methods. Furthermore, it is fundamental to constantly update epidemiological data of infection acquired by laboratory workers, to develop accurate risk indicators.

**Key words:** parasite; infection; resistance; prophylaxis; risk; biohazard.


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**Introduction**

Workers in clinical or research laboratories are exposed to multiple risks of infection and infestation, mainly through accidental exposure, which is not always recognised. In addition, laboratory workers often underestimate the risk and do not notify the exposure, making it difficult to collect reliable epidemiological data [1]. It was estimated that the biological risk for researchers is seven times higher compared to hospital and public-care workers [2]. In the early 1950s the annual incidence of infection or infestation, calculated as the attack rate, was 4.1 per 1000 among researchers [3]. However, epidemiological data on Laboratory Associated Infections (LAIs) are not exhaustive, in particular with regards to parasitological risk, the data are very fragmentary and outdated [2]. Therefore, the preventive measures to be taken remain uncertain and are not always implemented according to the appropriate work practices due to the low perception of risk by laboratory workers [4]. Handling specimens that may contain viable parasites potentially infectious requires a multi-faceted approach based on developing standard practices and focusing on the infectious agents to prevent their transmission.

Amid the lack of reliable estimation on the magnitude of LAIs, the control measures are mostly proposed on the basis of experience with one pathogen, on old data which is mostly scattered, and on the concept of hazard analysis and the knowledge derived from the transmission of a pathogen outside the research laboratories [5,6]. Risk management based on available information may not suffice in the current laboratory environments, however, it is the only code of information that laboratories must implement until the
systematic surveillance of LAIs. Safe laboratory operations are founded on the risk assessment of the pathogens as this process identifies the hazardous characteristics of the infectious agent and the likelihood/chances of exposure that can potentially cause an LAI and the consequences of acquiring such an infection [7].

The aim of this study was to collect all the data available in the literature and analyse the risk of the main zoonoses in the veterinary parasite diagnostic laboratory, in order to outline the potential exposure of the employees and draw up prevention measures. The study was divided into a special monographic part concerning zoonotic parasites among protozoa (Cryptosporidium spp., Entamoeba spp., Giardia duodenalis, Leishmania spp., Toxoplasma gondii) and helminths (Echinococcus spp., Toxocara canis, Ancylostoma caninum, Schistosoma spp., Strongyloides stercoralis), and then a specific part on LAIs and preventive measures to be taken in the laboratory.

Cryptosporidium spp.

General information

Cryptosporidium spp. is one of protozoan parasites which infect fish, amphibians, reptiles, birds and mammals [8]. The name of the disease is Cryptosporidiosis, which is a frequent cause of diarrhea in humans, especially in susceptible subjects like children and immunocompromised individuals [9,10]. It is primarily transmitted through water (Waterborne disease) but other mechanisms of transmission are ingestion with contaminated food, faecal contact, and direct contact with infected people [8,11]. The infection has been reported in more than 90 countries and on five continents [12] and Cryptosporidium was included in the World Health Organization (WHO) List of Neglected Diseases in 2004.

Cryptosporidium spp. is a coccidian parasite belonging to the Phylum Apicomplexa [13], Family Cryptosporidiidae [14]. This parasite has a monoxenous cycle and alternates an asexual phase with a sexual phase and finally a sporogenic phase with the formation of sporulate oocysts. To date, 30 species and 60 genotypes have been classified, but only two species, Cryptosporidium parvum, and C. hominis, frequently infect humans. In fact, C. canis, C. felis, C. meleagridis, C. muris, C. cuniculus, C. ubiquitum, and C. viatorum are potentially zoonotic, but rarely infect humans, while C. parvum is responsible for most of the infections in humans, with a significant impact both on human health and the health of domestic farm animals, as has not host specificity [12,15]. Infection begins with the ingestion of sporulated oocysts (the infectious dose is between 9 and 1024 oocysts) [16]. Oocysts may be thin-walled, or thick-walled. Thick-walled oocysts are responsible for the spread of infection, thanks to the high environmental resistance, determined by the presence of the double wall which is composed of hydrophobic proteins [17], while the thin-walled ones determine self and chronic infection [18,19].

Occupationally-acquired infections have occurred quite commonly in personnel working with this agent, especially if infected calves were the source of the oocysts, but other infected animals pose potential risks as well. Circumstantial evidence suggests that airborne transmission of oocysts via droplets of this small organism (i.e., 4–6 µm in diameter) might occur [7]. Sixteen cases of cryptosporidiosis are reported among LAIs [4].

Resistance in the environment and prophylaxis

Oocysts released into the environment can be infectious even for several months due to their protective external structure [20]. Oocysts suspended in deionized water at temperatures of 0 °C, 5 °C, 10 °C, and 20 °C remain infectious after 6 months, while those kept at a temperature between 25 °C and 30 °C are active for 3 months and remain infectious only for 1 week at 35 °C [21]. Oocysts can survive at -4 °C and 4 °C in the soil for more than 12 weeks, but at temperatures higher than 25 °C, degradation is more rapid (80% decrease after 7 weeks). The degradation of oocysts in bovine feces occurs after 4 weeks (80% decrease), probably due to drying and the presence of microorganisms, either in the feces or in the soil [22] (Table 1).

Oocysts can survive even in saltwater for a long time, some oocysts still remain active after 40 days in 35% salinity and at a temperature of 18 °C [23]. Humidity is a crucial factor for the survival of oocysts: only 3% of oocysts remained viable for two hours at room temperature and all oocysts died after this time [21, 24]. Exposure to UV radiation can affect the

| Table 1. Maximum period of viability of Cryptosporidium spp. oocyst [8,22,23]. |
|-------------------------------|----------------|----------------|
| Materials                     | Temperature    | Resistance of cyst (weeks) |
| Water                         | 0 °C / 20 °C   | 24              |
|                               | 25 °C / 30 °C  | 12              |
|                               | 35 °C          | 1               |
| Soil                          | -4 °C / 4 °C   | > 12            |
|                               | 25 °C          | 7               |
| Bovine feces                  | 25 °C          | 7               |
| Water with NaCl               | 18 °C          | > 7             |
| 35%                           |                |                 |
viability of oocysts; [24], report that this condition can lead to a 90% reduction of oocysts after 3 hours.

The ability to survive both wastewater treatments and drinking water treatments has been extensively studied for Cryptosporidium spp., as a factor of considerable epidemiological importance [20]. The classic processes of wastewater treatments such as chlorination are ineffective and on the other hand, traditional treatments of drinking water, such as coagulation, flocculation, and filtration, do not sufficiently reduce the presence of oocysts. The most effective treatments are of a new generation: microfiltration, reverse osmosis, ultrafiltration, use of UV combined with advanced oxidation, and UV disinfection with peroxide [25]. Environmental oocysts are also resistant to various chemical compounds such as sodium hypochlorite, denatured alcohol, 70%, and absolute ethanol [26]. Oocysts are inactive after exposure of only 2 hours to hydrogen peroxide and after 12 hours to 6% sodium hypochlorite [26]. Commercial disinfectants are not effective against Cryptosporidium spp. 3% hydrogen peroxide devitalizes Cryptosporidium oocysts after sufficient exposure. For this purpose, it is recommended to disinfect the surfaces to remove organic material followed by the application of 3% undiluted hydrogen peroxide for 30 minutes which can then be removed with absorbent paper and letting the surface dry for 10/30 min [7].

Entamoeba spp.

General information

Entamoeba spp. are protozoan parasites that form pseudopods and belong to the phylum Amoebozoa, class Archamoebae, and family Entamoebidae. The genus Entamoeba [27] includes different species that infect humans and different animals as reptiles, birds and amphibians, and others [27,28]. About seven species (E. histolytica, Entamoeba coli, Entamoeba hartmanni, Entamoeba polecki, Entamoeba dispar, Entamoeba moshkovskii, and Entamoeba bangladeshi) are able to inhabit the human intestine and one (Entamoeba gingivalis) that can be found in the oral cavity.

Although E. polecki and E. moshkovskii have occasionally been implicated as a cause of gastrointestinal symptoms, most Entamoeba species are generally commensal organisms of the gut, with the exception of E. histolytica, which is responsible for different health problems ranging from mild diarrhoea to invasive and extraintestinal infections. E. histolytica infects up to 50 million people worldwide, with nearly 100,000 deaths per year [29], primarily in developing countries such as Africa, India, and Central and South America [30]. The pathogenic E. histolytica and the non-pathogenic species E. dispar and E. moshkovskii form morphologically indistinguishable cysts with four nuclei averaging 10-16 µm in size [31]. Only molecular techniques allow for distinguishing commensal species from species with confirmed pathogenicity [32]. The morphological identity of the three species makes it difficult to differentiate pathogenic and non-pathogenic Entamoeba by microscopic examination, adding to the disease’s complexity. Previous statistics reported that 90% of E. histolytica cases were asymptomatic, and only 10% had some symptoms, resulting in an overestimation of the prevalence of human intestinal amoebiasis [33].

The cycle of infection begins when the cyst form of the organism is ingested in fecally contaminated food or water. The acid-resistant cyst passes unharmed through the stomach until it reaches the small intestine where it excysts to form eight trophozoites, the motile and invasive form of the species. Trophozoites migrate to the large intestine, where they may either colonize the bowel lumen as commensal flora or invade the colonic epithelium, causing inflammation and destruction of the bowel wall. What influences this decision to invade is as yet unknown, but potential factors include genetic differences between amoeba strains and host variations, such as intestinal flora, nutritional state, and immunocompetence. After the invasion, amoebae can enter the portal circulation and be transported to various target organs such as the liver, brain, and lungs. Extraintestinal amoebiasis is most commonly found in the liver [34].

Although ingestion of contaminated food or water is the most common transmission route, person-to-person transmission can also occur in settings with high crowding and poor personal hygiene, such as mental hospitals and daycare centers. In animal models, only 10-100 cysts are needed to cause amoebic dysentery, a contagious dose comparable to the notoriously contagious Shigella spp., which can be transmitted by as few as 10-100 organisms [35]. There is no notified Acquired Laboratory Infection of Entamoeba, although the possibility is real.

Resistance in the environment and prophylaxis

The cysts can survive days to weeks in the external environment. Since Entamoeba cysts are infectious when excreted, laboratory personnel should take routine precautions when working with stool specimens and fecally contaminated material, such as washing their hands thoroughly after handling specimens. Even
well-preserved specimens should be handled with caution because parasites in poorly preserved specimens may still be viable. Commercially available iodine-containing disinfectants, as well as high concentrations of chlorine (1 cup of full-strength commercial bleach [5% chlorine] per gallon of water [1:16, vol/vol]), are effective against *Entamoeba* spp. 

**Giardia duodenalis**

*Giardia duodenalis,* also known as *Giardia lamblia* or *Giardia intestinalis,* is the most common cause of parasitic diarrhea in the world [36]. Giardiasis (the name of the disease) has been included in the "Neglected Disease Initiative" project of the WHO since 2004, due to its prevalence and incidence in the poorest areas of the world [37]. For this reason, is discussed as the most important parasitic cause of travelers’ diarrhea, together with *Cryptosporidium* spp. 

Transmission occurs via the fecal-oral pathway, most frequently through the ingestion of contaminated food and water, making it also classified as "Foodborne disease" and "Waterborne disease" [38]. Furthermore, person-to-person and animal-person transmission is possible, although it is less prevalent [39].

*Giardia duodenalis* is considered a species complex, that includes 8 genetic groups or assemblages, identified with the letters from A to H. The assemblages A and B are subdivided into subgroups, and they are the only ones recognized as causing infection both in mammals and in humans, showing a zoonotic potential that cannot be underestimated [40].

*Giardia duodenalis* is a flagellated protozoan classified as diplomonads [41]. It has two stages: trophozoite, an active and vegetative form, and cyst which is the infectious form, relatively inert and resistant to the environment [42]. After ingestion of cysts, exposure to pancreatic enzymes and acidic pH of the stomach stimulates excystation with the release of two trophozoites, thus initiating the infectious process [42–44]. Trophozoites reproduce by binary fission in the crypts of the duodenal mucosa and in the proximal part of the jejunum; in the ileum, encystation takes place [45]. Its strong resistance to all external agents is due to the structure of the cystic wall which is composed of chitin [46] and the presence of three membranes: the outer membrane is linked to the filamentous elements of the cystic wall, the two inner membranes enclose the peritrophic space, where the innermost one is the plasma membrane of the trophozoite [47]. Unlike other parasites such as nematodes, *G. duodenalis* do not require maturation periods or activation after their excretion but are immediately able to infect a new host [48].

The parasite is monoxenous, has a simple and direct life cycle. Infection occurs after ingestion of the cystic form. The infectious dose for humans is between 10 and 100 cysts [45,49,50]. The infection can have a completely asymptomatic course to mild or severe course, even asymptomatic subjects eliminate cysts through feces. Age, immune status, co-infection with other protozoan parasites and microbiota of the host are factors that can influence the disease course. Children and immunocompromised individuals are considered to be at risk in which diarrhea can become chronic [11]. Laboratory could be infected through the wrong manipulation of stool specimens, the workers in the cleaning of disposable materials may be infected by accident. Nevertheless, among the LAIs, only two cases of giardiasis are reported [4].

**Resistance in the environment and prophylaxis**

Cysts of *G. duodenalis* have a marked resistance in the environment, sometimes to water purification treatments too, which allows the spread of infection through contaminated foods [51]. The resistance of cysts in the environment depends on climatic factors, such as temperature and humidity: they resist for about 77 days at 8 °C, from 5 to 24 days at 21 °C and about 4 days at 37 °C in distilled water [22,52] and remain mostly stables at 4 °C for 11 weeks in water, 7 weeks in soil and 1 week in bovine feces [22]. Analyses conducted at -4 and 25 °C in soil and faecal samples show that cysts are non-infectious after 1 week; in water, they are inactivated in less than 1 week at -4 °C and in 2 weeks at 25 °C [22] (Table 2). *Giardia* cysts are also sensitive to dryness and heat (> 40 °C) [53]; they show survival of 1 hour and a half in marine waters (salinity of 35 ppm) exposed to solar radiation, highlighting a significant role of salinity in their inactivation and also certain susceptibility to UV rays.

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**Table 2. Resistance of *Giardia* spp. cyst in water, soil and bovine feces [22].**

<table>
<thead>
<tr>
<th>Medium</th>
<th>Temperature</th>
<th>Resistance of cyst (per week)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>- 4 °C</td>
<td>&lt;1</td>
</tr>
<tr>
<td></td>
<td>4 °C</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>25 °C</td>
<td>2</td>
</tr>
<tr>
<td>Soil</td>
<td>- 4 °C</td>
<td>&lt;1</td>
</tr>
<tr>
<td></td>
<td>4 °C</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>25 °C</td>
<td>1</td>
</tr>
<tr>
<td>Bovine feces</td>
<td>- 4 °C</td>
<td>&lt;1</td>
</tr>
<tr>
<td></td>
<td>4 °C</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>25 °C</td>
<td>1</td>
</tr>
</tbody>
</table>
The UV could represent an effective medium for water disinfection [54]. The ability to survive potabilization treatments is one of the critical factors related to this protozoan. It has been widely reported that cysts can remain in the environment after a disinfection treatment, due to their physical and chemical structure. The classic disinfection processes – coagulation, flocculation, sedimentation, and filtration – are ineffective in eliminating Giardia spp. cysts [25]. Modern drinking water treatment systems, such as ozone treatment, reverse osmosis, UV disinfection combined with oxidation, microfiltration, and ultrafiltration are more effective compared to the classical processes [24]. In fact, Giardia cysts have a certain resistance to numerous chemical compounds: disinfectants such as chlorine and chloramines have proved to be ineffective against the protozoan, while they are sensitive to sodium hypochlorite [55].

The prevention of giardiasis is based on interventions of infection control and effective water purification. Hand hygiene assumes significant importance to reduce the chance of transmission between people, therefore, hand washing, proper disposal of waste (for example, diapers), and treatment of symptomatic people (especially children) can effectively prevent the spread in nurseries. Hand washing with soap and water is preferable to the use of sanitizing hand gel without rinsing, because of its effectiveness for the trophozoite form, but not for the cyst [56]. The disinfection of tools and laboratory surfaces can be done through a 5% chlorine concentration solution which is effective against G. duodenalis [4].

Toxoplasma gondii

General information

Toxoplasma gondii is one of the most widespread parasites in the world, both in warm-blooded animals and humans [57]. Toxoplasmosis is one of the most important foodborne parasitic zoonoses, occupying the fourth place in the global ranking of food-borne parasites drawn up by the Food and Agriculture Organization of the United Nations (FAO) and the WHO [70,71]. Toxoplasma is a protozoan belonging to the phylum Apicomplexa, family Sarcocystidae and have only one genus Toxoplasma [58], and one species, namely T. gondii [59]. Three main genetic lineages - type I, II and III - isolated in North America, Europe, and Africa, together with a high percentage of atypical genotypes have been so far identified. Type II is the most common followed by type III and atypical genotypes in farm animals while type I is rarely found in these animals [60]. Type II strains have a high capacity to produce cysts in animal models and are frequently associated with infections in agricultural animals, instead type III strains appear to be more common in animals, although in general they are not associated with the disease. Type I strains, although rare in animals, have shown increased prevalence in some cases of congenital infection and in AIDS patients, suggesting that they are more likely to cause disease in humans [61]. In addition, there are new strains that show unexpected virulence features in humans [62]. All warm-blooded animals can be the intermediate hosts, including farm and wild animals, as well as humans, while the definitive hosts are represented by the felids, especially the cat [63]. In the intermediate hosts, infection occurs through the ingestion of tissue cysts or sporulated oocysts and two stages of asexual development of the parasite take place [63]. In the definitive host felids, both the asexual and sexual reproduction phases take place. The cat, through the feces, releases up to 100 million oocysts which do not sporulate until about 15 days in the external environment [64], eventually undergoing sporulation and becoming infectious within 1-5 days [65]. The parasite is transmitted mainly through the ingestion of tissue cysts in raw or undercooked meat, or through oocysts expelled from the cat with the feces that had time to sporulate and consequently become infectious. Other possible transmission routes are water, blood transfusions or organ transplants, and vertical transmission from mother to child [4].

Laboratorians can become infected through ingestion of sporulated oocysts from feline fecal specimens or through the skin or mucosal contact with either tachyzoites or bradyzoites in human or animal tissue or culture. All T. gondii isolates should be considered pathogenic for humans even if they are avirulent for mice [66]. Forty-seven laboratory-acquired cases of T. gondii infection have been reported [4].

Resistance in the environment and prophylaxis

The infectious sporulated oocysts have a high environmental resistance: they can survive in the soil for one year and a half [67], in water at a temperature of 4 °C for four and a half years, and at 20-25 °C for six months [68]. In marine waters with a salinity of 15 %, sporulated oocysts survive for at least 24 months [69]. The resistance depends on the oocyst’s stage of sporulation: for example, exposure to 37 °C for 24 hours is lethal for unsporulated oocysts, while sporulated oocysts survive at least 32 days at a
temperature of 35 °C and 9 days at 40 °C [68]. At a temperature of 4 °C, unsporulated oocysts are inactivated after 6-10 weeks [70] (Table 3).

Tissue cysts, on the other hand, are inactivated by cooking or freezing, respectively at 66 °C and -12 °C in less than one second [71,72]. Under conditions of salinity equivalent to 3.3% NaCl, tissue cysts survive for at least 21 days at 10 °C, 14 days at 15 °C, and 3 days at 20 °C. At NaCl concentrations of 6%, tissue cysts do not survive when exposed for 7-14 days [73].

Cases of toxoplasmosis from the consumption of drinking water have been documented, which highlights the ineffectiveness of the chlorination of water for human consumption. In fact, under laboratory conditions, 4-hour treatments at different concentrations of chloramines, free chlorine, or chlorine dioxide, are ineffective against T. gondii, as well as treatment with ozone. Sporulated oocysts maintain their viability even after exposure to 100 mg/L of free chlorine for 30 min and for 2, 4, 8, 16, and for 24 hours, as well as to 6 mg/L of ozone for 1, 2, 4, 8, and 12 minutes and to 9.4 mg/L of ozone for 20 minutes [74]. UV treatments, on the other hand, have a considerable effect leading to the inactivation of 99.9% of sporulated oocysts [75]. The data obtained are attributable to the particular structure of sporulated oocyst which protects the sporozoites from chemical damage of acids, solvents, and other oxidizing elements. The disinfection efficacy was demonstrated at exposure at concentrations of 10% and 5% ammonium hydroxide for 10 and 30 minutes respectively [76]. However, ammonium hydroxide is a potent toxicant and presents concrete health risks [77]. The laboratory instruments and glassware contaminated with T. gondii oocysts must be sterilized through the use of heat [4].

**Leishmania spp.**

**General information**

*Leishmania* (Kinetoplastida, Trypanosomatidae) is a genus of protozoan parasites that are transmitted by the bite of blood-sucking female phlebotomine sandfly (Diptera, Psychodidae). Leishmaniasis is one of the most significant of the neglected tropical diseases, with 350 million people in 88 countries worldwide living at risk of developing one of the many forms of the disease [78]. The *Leishmania* infection occurs when sandflies ingest amastigote forms of the parasite while feeding on a reservoir host, and then, during another blood feeding, the sandfly regurgitates metacyclic promastigotes into the host [78,79].

### Table 3. Resistance of *Toxoplasma gondii* oocysts.

<table>
<thead>
<tr>
<th>Medium</th>
<th>Temperature</th>
<th>Resistance of cyst (sporulate)</th>
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</thead>
<tbody>
<tr>
<td>Water</td>
<td>4 °C</td>
<td>54 months</td>
</tr>
<tr>
<td></td>
<td>20 – 25 °C</td>
<td>6 months</td>
</tr>
<tr>
<td>Water with NaCl</td>
<td>35 °C</td>
<td>24 months</td>
</tr>
<tr>
<td>15 %o</td>
<td>40 °C</td>
<td>32 days</td>
</tr>
<tr>
<td></td>
<td>- 4</td>
<td>9 days</td>
</tr>
</tbody>
</table>

About 20 species of *Leishmania* infect mammals and many of them caused human leishmaniasis [80], with different clinical outcome based on the species. Human Cutaneous Leishmaniasis (CL) is caused by most *Leishmania* species in the subgenus *Leishmania*, such as *Leishmania major* from Africa and Asia, and *Leishmania mexicana* from Central and South America, and by many species in the subgenus *Viannia*, which are restricted to Latin America (for example *Leishmania brasiliensis*). Mucosal leishmaniasis (ML) is caused by *Leishmania tropica*, *Leishmania major*, *Leishmania infantum*, *Leishmania donovani* in the Old World (Africa, Europe, Asia), while several species of *Vianna* subgenus can cause ML in the New World (The Americas) [81]. Any parasite causing cutaneous or mucosal leishmaniasis can visceralize, but only two species of the subgenus *Leishmania* routinely do so, and these are the causative agents of most human Visceral Leishmaniasis (VL) worldwide, that are *Leishmania donovani* and *Leishmania infantum* [82]. *Leishmania infantum* is the zoonotic form, with dogs as main reservoir, occurs in the Mediterranean basin, China, the Middle East, and South America. *Leishmania donovani* is the anthroponotic form, with human-to-human transmission without animal reservoir. This form is prevalent in East Africa, Bangladesh, India, and Nepal [82].

The main three phenotypic categories of *Leishmania* disease are cutaneous, mucosal, and visceral leishmaniasis, but CL and VL are the most severe clinical forms of the disease [81,83]. In the CL, the first sign of an infection is typically a small erythema that develops at the site where an infected sandfly has bitten the host. The erythema develops into a papule, then a nodule that progressively ulcerates over a period of 2 weeks to 6 months to become the lesion that is a feature of CL. Resolution of the disease results in a lifelong cutaneous scar, which, depending on its size and location, may cause substantial trauma in affected individuals [84]. The most lethal form of leishmaniasis, VL (also known as kala-azar) can cause systemic infection affecting the liver, spleen, hematogenous and lymphatic system [83]. The disease is progressive and a symptomatic infection left
untreated is generally fatal, with a mortality rate of 75-95%. Death usually occurs within 2 years, although spontaneous cures may occur [82]. Asymptomatic infection represents approximately 20–60% of *Leishmania* spp. infection in endemic areas [83]. Currently, no vaccination against leishmaniasis is available for humans. The primary prevention is based on the management of animal reservoir host and control of the sandfly population [85]. However, deforestation, agricultural practices, and urbanization have led vectors to feed on human beings rather than synanthropic reservoirs [84]. Furthermore, with climate change, the incidence and geographical distribution of leishmaniasis is expected to increase [81]. Secondary and tertiary prevention is dependent on the optimum management of cases and may be assisted by the use of clinical guidelines [85].

Fourteen cases of LAIs caused by *Leishmania* spp. have been reported, due to negligence (e.g., during mouth pipetting, re-capping a needle), accidental percutaneous exposure, and needlestick injury [4].

**Resistance in the environment and prophylaxis**

Studies conducted in vitro shown that viscerotropic *L. tropica* survived as intracellular parasites in monocytes for 25 days in the red blood cell fraction kept at 4 °C, five days in the platelet fraction kept at 24 °C, 35 days in the red blood cell fraction frozen with glycerol and for 30 days in unprocessed whole blood left at 4 °C. Identical experiments with *L. donovani* resulted in comparable survival data. Intracellular parasites survived longer than did stationary phase extracellular promastigotes or free amastigotes [86]. For this reason, blood specimens should be handled with care, as well as needles and sharp objects. Data on the resistance to different temperatures of *Leishmania* in the blood are given in Table 4.

**Echinococcus spp.**

**General information**

*Echinococcus* spp. is a Taeniidae cestode parasites, the genus *Echinococcus* represents a group of nine species, but only two are important for public health: *Echinococcus granulosus*, which causes cystic echinococcosis (CE), and *Echinococcus multilocularis* which is responsible for alveolar echinococcosis (AE). *Echinococcus vogeli* and *Echinococcus oligarthra*, neotropical species, cause polycystic echinococcosis in tropical areas (Central and South America) while two other species have recently been identified: *Echinococcus shiquicus* in small mammals of Tibet and *Echinococcus felidis* in African lions. The zoonotic potential of the latter two species is still unknown [87]. The CE is a zoonosis caused by cestodes belonging to the species complex *Echinococcus granulosus sensu lato* (s.l.) [88]. This metacstodosis has a cosmopolitan distribution and represents a significant public health problem in different regions of the world [89]. Despite the efforts made in the field of prevention and control, WHO includes echinococcosis among the 17 neglected tropical diseases (NTDs) [90,91]. The presence of extensive sheep farms, the consistent presence of stray or shepherd dogs, unsupervised home slaughter, and improper disposal of carcasses are the predominant factors for the persistence of CE in endemic region [92]. The biological cycle of *Echinococcus* spp. is indirect and requires two hosts, a definitive and an intermediate host. The adult form of *E. granulosus* resides in the small intestine of the definitive host, represented mostly by the dog [87], while the intermediate host is mainly sheep, but also buffalo, horses, cattle, pigs, camels, and cervids. The ingestion of eggs by the intermediate host leads to the release of oncospheres, which migrate through the circulatory system into various organs, especially in the liver and lungs, developing hydatids. The definitive host becomes infected by ingesting the visceral organs of the intermediate host infected with hydatids [93]. Humans, occasional/accidental intermediate hosts, become infected by ingesting eggs that can contaminate water and plants and can develop hydatids in different organs. Transmission of *E. multilocularis* occurs in a sylvatic cycle, which is sometimes linked via infected small mammals to domestic dogs and cats. In the sylvatic cycle, foxes play a key role as definitive hosts, and small mammals, mainly rodents, are the intermediate hosts. However, dogs and cats can also serve as competent definitive hosts [93,94]. *Echinococcus multilocularis* (the small fox tapeworm) is widely distributed within but restricted to the Northern Hemisphere [93].

Several studies indicate in humans a higher frequency of CE in the liver rather than in the lungs (average rate 2.5:1), and even less frequent cysts are detected in the spleen, kidneys, heart, bones, and central nervous system (CNS) [87,95]. Concerning AE, data from patients with single-organ involvement indicate

<table>
<thead>
<tr>
<th>Table 4. Resistance of <em>Leishmania</em> spp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium</td>
</tr>
<tr>
<td>----------------------------------------</td>
</tr>
<tr>
<td>Red blood cell fraction</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Unprocessed blood</td>
</tr>
<tr>
<td>Red blood cell fraction with glycerol</td>
</tr>
</tbody>
</table>

that initially establish almost exclusively in the liver (approximately 99% of the cases) and is rarely found in extra-hepatic sites [94]. The infection in the initial phase is always asymptomatic, and for some patients, it might remain asymptomatic for years (the incubation period vary between less than 5 and up to 15 years for AE) or for the entire life or manifests in different clinical form depending on the number, size, and stage of development of metacestode, of the organ involved, the localization of hydatid cysts (if they cause pressure in adjacent tissues and organs) and the host defense mechanisms [94]. Clinical symptoms usually appear after several months or years. Liver cysts can cause pain in the abdominal region, hepatomegaly, cholestasis, biliary cirrhosis, and ascites, and in the rare case of metacestode ruptures, anaphylaxis might occur. In the case of pulmonary cysts, the most frequent signs and symptoms are chronic cough, dyspnea, hemoptysis, pleurisy and lung abscesses. Larval growth in the bones can cause pain in the affected area, muscle weakness, bone deformities, and even fractures. The development of the cyst at the cerebral level is atypical as well and causes extensive erosion of the bone. The development of metacestode, of the organ involved, the localization of hydatid cysts (if they cause pressure in adjacent tissues and organs) and the host defense mechanisms [94]. Clinical symptoms usually appear after several months or years. Liver cysts can cause pain in the abdominal region, hepatomegaly, cholestasis, biliary cirrhosis, and ascites, and in the rare case of metacestode ruptures, anaphylaxis might occur. In the case of pulmonary cysts, the most frequent signs and symptoms are chronic cough, dyspnea, hemoptysis, pleurisy and lung abscesses. Larval growth in the bones can cause pain in the affected area, muscle weakness, bone deformities, and even fractures. The development of the cyst at the cerebral level is atypical as well and causes extensive erosion of the bone.

**Resistance in the environment and prophylaxis**

The eggs of *Echinococcus* spp. released into the environment are already infectious and easily spread through insects and birds. In the environment, they remain viable for a variable period, depending on temperature and humidity conditions. Eggs of *E. multilocularis* remain infective for approximately 1 year in a suitable, moist environment at lower temperatures, but they are sensitive to desiccation and high temperatures. Their high resistance to low temperatures is a precondition for their survival in Arctic regions [96]. Both *E. granulosus* and *E. multilocularis* eggs can survive at 50 °C for 24 hours but are killed at 70 °C within 96 hours and at 80 °C to 83 °C within 48 hours. Deep-freezing at -70 °C for at least 4 days or at -80 °C for at least 2 days is recommended for inactivating *E. multilocularis* eggs in carcasses or intestines of final hosts or in fecal material before examination in the laboratory [94,97]. At 4 °C, eggs can remain infectious for over 300 days, but the viability is significantly reduced in case of a rise in temperature. Eggs are devitalized within 2-14 days at a temperature range of 37-39 °C [98]. The eggs are sensitive to heat, the inactivation begins at temperatures of 50 °C and completes at 60 °C; 10 minutes at 72 °C is the most effective method for the destruction of eggs [99,100]. The eggs of *Echinococcus* spp. are sensitive to drying. At a relative humidity of 25% eggs of *E. granulosus* were killed within 4 days and at 0% within 1 day. Eggs of *E. multilocularis* lost infectivity to rodents after exposure at +25 °C and relative humidity (RH) of 27% for 2 days, at + 43 °C and 15% RH for 2 hours, and at +45 °C and 85%-95% RH for 3 hours [96]. Data on the resistance to different temperatures and relative humidity of *Echinococcus* are given in Table 5. The exposure to 0.7% sodium hypochlorite for 7 minutes at 25–27 °C is effective for egg inactivation [100], but sodium hypochlorite solution (NaOCl) at a minimum concentration of 3.75% in water disrupts the embryophores of *Echinococcus* spp. eggs and damages the majority of the oncospheres within a few minutes. Finally, the eggs of *E. granulosus* retained viability in ethanol (50%, 70%, 95%) after 5 min to 60 min exposure [96]. The disinfection of workbenches and equipment can be carried out with commercial bleach, which contains 50 g/l of free chlorine and must be diluted 1:10 to obtain 5.0 g/l, which is effective *E. granulosus* [4].

**Schistosoma spp.**

**General information**

Schistosomiasis is a water-borne infectious disease caused by blood flukes of the genus *Schistosoma* that affects humans and domestic and wild animals in many tropical and subtropical regions. Human schistosomiasis, included by the WHO in the list of “Neglected Tropical Diseases”, is mainly caused by *Schistosoma haematobium*, *Schistosoma mansoni* and *Schistosoma japonicum*, whereas *Schistosoma guineensis*, *Schistosoma intercalatum* and *Schistosoma mekongi* have lower global prevalence [101]. After malaria, schistosomiasis is the most common parasitic disease in humans. It is currently endemic in over 70 tropical and subtropical countries, where it is thought to affect more than 240 million people. Additionally, it

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**Table 5. Resistance of Echinococcus spp.**

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Relative humidity (RH) (only for <em>E. multilocularis</em>)</th>
<th>Time of viability</th>
</tr>
</thead>
<tbody>
<tr>
<td>-80 °C</td>
<td>-</td>
<td>2 days</td>
</tr>
<tr>
<td>-70 °C</td>
<td>-</td>
<td>4 days</td>
</tr>
<tr>
<td>4 °C</td>
<td>-</td>
<td>300 days</td>
</tr>
<tr>
<td>37 °C – 39 °C</td>
<td>-</td>
<td>2-14 days</td>
</tr>
<tr>
<td>50 °C</td>
<td>-</td>
<td>24 hours</td>
</tr>
<tr>
<td>72 °C</td>
<td>-</td>
<td>10 min</td>
</tr>
<tr>
<td>25 °C</td>
<td>27%</td>
<td>2 days</td>
</tr>
<tr>
<td>43 °C</td>
<td>15%</td>
<td>2 hours</td>
</tr>
<tr>
<td>45 °C</td>
<td>85% - 95%</td>
<td>3 hours</td>
</tr>
</tbody>
</table>
poses a threat to an additional 780 million people who live in areas where they are susceptible to infection [102].

Despite, to our knowledge, there is no information on the current prevalence of animal schistosomiasis, in the past it affected more than 165 million animals and was widely distributed throughout Africa, the Mediterranean Basin, and Southwestern Asia [103]. At least ten different Schistosoma species have the potential to cause the illness, with Schistosoma bovis standing out due to its pathogenicity for domestic ruminants [104].

The zoonotic potential of S. bovis and its effects on human health have been recently revealed. Indeed, the occurrence of S. bovis x S. haematobium hybrids in human urine and stool samples has been described in people from several villages along the Senegal River Basin in Northern Senegal [105] as well as in a recent outbreak of urogenital human schistosomiasis that occurred in Corsica [106].

Other Schistosoma species that parasitize birds and mammals can also cause cercarial dermatitis in humans, but this is clinically distinct from schistosomiasis [107].

In general, Schistosoma spp. eggs are eliminated through feces or urine, depending on the species. When the eggs hatch, they release miracidia, which swim and penetrate specific snail intermediate hosts. The stages in the snail include two generations of sporocysts and the production of cercariae. When the infective cercariae are released from the snail, they swim, penetrate the skin of the vertebrate host (humans or animals), and shed their forked tails, transforming into schistosomulae. They migrate through the venous circulation to the lungs, then to the heart, and finally to the liver, where they mature and exit through the portal vein system. Male and female adult worms copulate and live in the mesenteric venules, which vary by species. Females lay eggs in the portal and perivesical systems' small venules. The eggs are gradually moved toward the lumen of the intestine or the bladder and ureters, where they are eliminated with feces or urine [108].

Such cercariae, which swim freely, could infect laboratories working with aquaria for snail intermediate hosts; dissecting or crushing infected schistosome-infected snails could also expose workers to droplets containing cercariae. Therefore, workers performing such tasks ought to use gloves. Furthermore, people at risk of schistosomiasis should wear a long-sleeved gown or coat and shoes rather than sandals to reduce the amount of exposed skin. So far, at least nine laboratory-acquired cases of schistosomiasis have been reported in workers who came into contact with infected snails while not wearing protective clothing [1,109].

Resistance in the environment and prophylaxis

Environmental factors, particularly those affecting the intermediate host snail, play a significant role in transmission. Variations in the weather conditions, such as alterations in temperature, rainfall/precipitation, flood, drought, and pH among others, have been recognized to have a significant impact on the lifespan and fecundity rate of both snails and the penetration of cercariae into the skin of the definitive host [110].

Concerning the prevention measures, travellers should be aware of the possibility of infection when engaging in activities that involve direct contact with water in endemic areas [111]. People who are inadvertently exposed to potentially contaminated water (such as by falling into a river) should vigorously dry off with a towel to try to remove any parasites before they penetrate the skin.

However, avoiding water contact may be extremely challenging, if not impossible, for residents of rural areas where schistosomiasis is endemic. Schistosomiasis is a disease associated with poverty, and although it can be prevented, it is frequently not present [112]. Preventive measures should include access to clean water and sanitary facilities. The risk from occupational exposure, such as fishing in rivers and lakes, will also be little affected by these measures, even though these pursuits are frequently the sole or primary source of income for poor families [113].

Furthermore, schistosomiasis can be prevented by using molluscicides in freshwater, but it can be challenging, expensive, and environmentally hazardous. In endemic areas, widespread praziquantel treatment and education campaigns are used to control the disease. Despite intensive development efforts, currently, no schistosomiasis vaccines are still available [114].

Toxocara spp.

General information

Toxocara is an important ascaridoid genus containing species of human and animal health significance [115]. The genus contains 21 species, but Toxocara canis and Toxocara cati are mainly responsible for human toxocariasis. The final hosts are the dog and the cat respectively, while the other animals, upon which dogs and cats usually prey, like rodents, are the paratenic hosts [116,117]. Humans represent an accidental host [118]. These parasites present a complex biological cycle with different...
possible infestation routes based on the age and immunological status of the definitive host consumption of raw or undercooked meat containing encysted larvae of *Toxocara* spp., coming either from paratenic hosts or directly from the environment, which migrate within the body and are encysted in several muscles and organs [117,119].

Toxocariosis in humans occurs predominantly with asymptomatic or subclinical symptoms, but symptomatic infestations can generate visceral *larva migrans* (VLM) syndromes, ocular *larva migrans* (OLM), or neurotoxocariosis [117]. VLM is typical in children aged 1 to 7 years of age, but can also be found in adults. Symptomatic infestations present with fever, gastrointestinal symptoms, hepatomegaly, abdominal pain, loss of appetite, and weight loss [117,120]. OLM occurs mainly in children aged 5 and 10 years, and the syndrome typically manifests itself with unilateral vision accompanied by occasional strabismus [117,121]. Instead, neurotoxocariosis presents a symptomatology affecting the CNS, but the frequency and location of *Toxocara* spp. in the CNS in humans remain unknown [117,122,123]. The different species of *Toxocara* are widespread throughout the world with higher concentrations in areas with a high population of domestic dogs and cats. However, toxocariosis is predominant in tropical and subtropical regions and in developing countries [124]. There is no reported case for *Toxocara canis*, but despite this, is crucial to pay attention because ascarid eggs are sticky, then contaminated laboratory surfaces and equipment must be thoroughly continuous cleaning to prevent worker exposure.

Resistance in the environment and prophylaxis

*Toxocara* spp. eggs remain infectious under different environmental conditions. In fact, the larva is well protected by the wall that surrounds it, composed of chitin and fibrous layers [124], which is resistant to different chemical agents such as formalin and different inorganic acids [117]. Under suitable environmental conditions (15–35 °C temperature, 85% relative humidity) the larva develops inside the egg after 2–5 weeks and is potentially infectious for paratenic and definitive hosts [125]. The eggs are inactivated at -15 °C and are sensitive to direct sunlight, although they can survive in favorable climatic conditions for 6 years [124]. Sodium hypochlorite at 7% is the most effective and economical disinfectant against *Toxocara* spp. eggs [124–126]. It is crucial to follow some important prevention rules, including the control of the hygiene of environments designed for animals, for example using sodium hypochlorite and exposing them to direct sunlight, and applying good health and hygiene practices. Data on the resistance to different temperatures and relative humidity of *Toxocara* are given in Table 6.

**Table 6. Resistance of *Toxocara* spp.**

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Relative humidity</th>
<th>Resistance of larvae</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 °C – 35 °C</td>
<td>85%</td>
<td>2-5 weeks (time to develop inside the egg) – 6 years</td>
</tr>
<tr>
<td>- 15 °C</td>
<td>-</td>
<td>Inactivate</td>
</tr>
</tbody>
</table>

**Ancylostoma spp. and Uncinaria stenocephala**

**General information**

*Ancylostoma caninum, Ancylostoma braziliense, Ancylostoma ceylanicum* and *Uncinaria stenocephala* are the parasites completing their lifecycles with dogs as the definitive hosts, and in the case of *A. braziliense, A. ceylanicum*, and *U. stenocephala* the cats as well [116]. Defined as "hookworms" for their morphology, they belong to the Order Strongylida, Family Ancylostomatidae [127].

Their biological cycle in the hosts is of direct nature. The host acquires the infection through the free-living L3 larvae, which can be taken either orally, through the predation of paratenic hosts, or through percutaneous penetration [7]. The infestation of puppies by *A. caninum* through transplacental and transmammary routes has also been documented [116,128,129]. *Uncinaria stenocephala*, also called "northern Ancylostoma", tolerates harsh climates and is present throughout Europe. *Ancylostoma caninum* is widespread mainly in central and southern Europe, while *A. braziliense* is widespread in tropical and subtropical countries and *A. ceylanicum* is distributed in Asia [130,131].

Apart from infection in animals, these parasites also infect humans which are the accidental hosts because they do not play any role in the completion of the biological cycle. In humans, the infectious larvae (L3), penetrate through the skin causing cutaneous *larva migrans* (CLM) also called "creeping eruption" which causes intense itching with serpiginous linear skin lesions having erythematous-papulo-vesicular appearance, which can grow a few millimeters per day [64]. If left untreated, the parasitic forms resolved spontaneously from one to two months, till larvae die. In rare cases, the larvae migrate into the intestine causing eosinophilic enteritis with abdominal pain, anorexia, diarrhea, and nausea [132-134]. An exception is represented by *A. ceylanicum*, which does not lead to CLM, but develops as an adult in humans, and is...
capable of causing chronic infestation and anemia, even in healthy individuals [135,136]. CLM is a frequent disease in subtropical countries, including the southern states of the USA, due to fecal contamination of the soil by infested animals [133,142]. Autochthonous cases of *A. caninum* have been reported in Europe and specifically in the UK, Germany, Italy and Serbia [135]. Only one case of LAIs caused by *Ancylostoma* spp. has been reported [4].

**Resistance in the environment and prophylaxis**

In an environment like the grass, the larvae of *A. caninum* survived best at a temperature range of 0 °C to approximately 20 °C. To kill larvae is recommended that sunlight be allowed to be present at least 2 hours every day on areas where there might be they are present. Is recommended to follow some prevention measures such as collection of dog and cat feces, regular cleaning of the litter, avoid walking barefoot, even on the beach, and use of sunbeds instead of towels when in direct contact with the sand [135]. The iodine concentrations of 70 ppm have been shown to kill infective larvae of *A. caninum* immersed in an aqueous iodine solution for one to five minutes [7].

**Strongyloides stercoralis**

**General information**

*Strongyloides stercoralis* is a zoonotic parasite, widespread throughout the world, which infests dogs, cats, and primates including humans [137,138]. *Strongyloides stercoralis* is one of the Soil Transmitted Helminths (STH) and is listed among the NTDs [133]. *Strongyloides stercoralis* (Rhabditida: Strongyloidae) has a biological cycle that can include a parasitic life phase and a free-living phase, depending on climatic conditions. Rhabditiform larvae of *S. stercoralis* are excreted through the feces of an infected individual and develop into filariform larvae (L3) that can infect a new host through the percutaneous or oral route. Larvae reach the pulmonary capillaries, penetrate the alveoli and pass through the larynx and pharynx finally reaching the small intestine after swallowing to mature into adults. Through the oral route, the larvae of *S. stercoralis* follow the same cycle, penetrating first through the intestinal mucosa and then carrying out the migration described above. Adult females deposit about 50 unfertilized eggs daily, which hatch in the intestinal wall, migrate into the intestinal lumen and are excreted through the feces. Sometimes the larvae penetrate the wall of the colon or the skin of the perianal area, establishing a self-infection that leads to a chronic form of infestation or spread into other organs. In the latter case, the infestation can be fatal [139,140]. In dogs, transmission to puppies by the galactogen route is also followed [129]. Strongyloidiasis in humans includes a number of nonspecific gastrointestinal symptoms such as diarrhea, abdominal pain and urticaria. However, most infestations, including chronic ones, remain asymptomatic. Asymptomatic infestations can be dangerous in the case of immunosuppressive treatments, especially with corticosteroids, because they can lead to disseminated infestation [140]. LAIs with *Strongyloides stercoralis* have been reported; furthermore 4 cases of *Strongyloides* spp. laboratory infections acquired from infected animals have also been reported [4].

**Resistance in the environment and prophylaxis**

Positive samples of *S. stercoralis* stored at 4 °C for 24, 48, and 72 hours were reexamined, which still had viable larvae after 72 hours of refrigeration [141]. Iodine concentration of 50 ppm kill the infective *S. stercoralis* larvae in 5 minutes, in vitro exposure to 70% ethanol has been shown to kill infective larvae within 3-5 minutes [7].

**LAIs – Laboratory Associated Infections**

Laboratory acquired infections (LAIs) include all infections associated with laboratory work carried out in clinical laboratories, research, teaching (medical and veterinary), and production facilities [2]. Both symptomatic and asymptomatic (subclinical) infections are comprised among the LAIs. Although these phenomena are not new in laboratories, the information collected, through publications and questionnaires, mainly considers symptomatic infections and associated symptoms, with minimal data on asymptomatic cases. In addition, in some cases, it is difficult to establish if the infectious disease is caused by a microorganism present only in the laboratory or even in the community. All these elements lead to assert that it is impossible to establish a real incidence of LAIs [2]. To date, there is no centralized system for reporting infections in the laboratory, therefore, epidemiological studies represent an indispensable tool to assess the nature and extent of this phenomenon.

In a review conducted by Herwaldt [4], 47 cases of *T. gondii* were reported from 1940 until the 90s, among which 23 were from the USA and 20 from Europe. In the majority of cases, infection occurred through parenteral exposure (through sharp injuries) followed by cases due to ingestion of oocysts and contact with mucous membranes by laboratorians. Of the 47 cases reported, only 9 were asymptomatic, the other subjects
had symptoms such as fever, headache, general malaise and lymphadenopathy, in 4 cases encephalitis had occurred and in two of these cases, myocarditis was reported. There was only one lethal case in 1951 caused by encephalitis and myocarditis [142]. Despite the infrequency of LAIs, the laboratory staff must remain cautious as many exposures remain unrecognized and accidental incidents may happen.

Fourteen cases of Leishmania spp. infection have been reported during the decades among 1930 – 2005: five cases of L. donovani and one case of Leishmania chagasi (considered same species of L. infantum in the Old World), three cases of Leishmania braziliensis, two cases of Leishmania tropica, one case of Leishmania mexicana and finally one case of Leishmania guyanensis [4]. All the infections were symptomatic, and only one person infected by L. donovani species complex developed manifestations of visceral involvement. In fact, although both L. donovani and L. chagasi are typically considered etiologic agents of visceral leishmaniasis, both can also cause cutaneous infection. Laboratory that developed CL had symptoms such as nodules, papular lesions at the site of exposure, someone also lymphadenopathy, and lymphangitis. Fortunately, none of these cases was fatal. In laboratory settings, leishmaniasis could be acquired through inadvertent contact with an infected sand fly, like natural route exposure, but transmission could also occur through contact with cultured parasites or specimens from infected persons or animals. In fact, nine of the reported cases were caused by parenteral exposure (e.g., needlestick injury), two cases by biting of infected animals, one case due to nonintact skin, one case by mouth pipetting (mucosal contact), and finally one accident was not recognised [4].

In the case of intestinal protozoa, very few case reports are available among both researchers and healthcare professionals: 2 cases of giardiasis and 16 cases of cryptosporidiosis have been reported to date, probably because these infections can be diagnosed and treated easily and also the disease is typically gastrointestinal rather than systemic [4]. Even in our laboratory, unfortunately, despite all the biosecurity precautions undertaken, a student working on the epidemiology of cryptosporidiosis of calves from 1995-1996, manifested several episodes of transient diarrhea attributable to these protozoa, as demonstrated by the analyzes carried out during the last episode (Scala, personal communication).

Four cases of helminth infestations reported so far from the scientific research laboratories were attributable to S. stercoralis, all contracted through skin contact, and only one has been attributed to Ancylostoma spp. [4]. Furthermore, nine laboratory-acquired cases of schistosomiasis have been so far reported [1]. The lack of reports reflects the fact that helminth infestations are generally less common than protozoan infections in laboratory environments. In fact, the use of gloves and laboratory coat, together with appropriate organizational measures (decontamination and disinfection) are able to stem the possibility of infestation.

Although the epidemiological information collected is not exhaustive, it is clear that the adoption of adequate biosecurity measures is necessary to prevent infections and infestations. It is necessary to implement information and training of personnel, good hygiene practices, the correct use of Personal Protective Equipment (PPE) and Collective Protective Equipment, and the improvement of the work organization and facilities. In fact, it is evident that the lack of adequate control plans, programmed for parasitology laboratory staff certainly leads to a considerable underestimation of the real cases of "parasitic" LAIs. For this reason, it is advisable to create a surveillance system, providing periodical check on the lab workers, with simple and

<table>
<thead>
<tr>
<th>Group</th>
<th>Effects on humans</th>
<th>Risk to workers</th>
<th>Prophylactic and therapeutic measures</th>
<th>Example of BA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risk Group 1</td>
<td>Low chances of causing disease</td>
<td>Very low</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Risk Group 2</td>
<td>Can cause diseases</td>
<td>Low risk; little chance of spreading in the community</td>
<td>Normally available</td>
<td>Giardia duodenalis; Entamoeba hystolytica Cryptosporidium spp; Toxoplasma gondii; Toxocara spp.; Strongyloides spp.; Ancylostoma spp.</td>
</tr>
<tr>
<td>Risk Group 3</td>
<td>Capable of causing serious illness</td>
<td>Serious risk; manage to spread in the community</td>
<td>Normally available</td>
<td>Escherichia coli (es. O157:H7 or O103); E. granulosus; E. multilocularis; E vogeli Leishmania brasilensis; Leishmania donovani</td>
</tr>
<tr>
<td>Risk Group 4</td>
<td>Serious illnesses</td>
<td>Serious risk; can spread very easily in the community</td>
<td>Normally not available</td>
<td>Congo–Crimea hemorrhagic fever; Lassa Virus; Ebola Virus</td>
</tr>
</tbody>
</table>
cheap tests. For example, seroprevalence of IgG against *G. duodenalis*, *Cryptosporidium* spp. and *Toxoplasma gondii*, or Portable US and dot-ELISA for CE surveys that have been demonstrated applicable, with a few limitations [143]. In addition, it is recommended to notify any cases of suspected transmission of infections to the occupational physician, to set up the appropriate measures of prophylaxis and control of infection.

**Biosecurity measures**

One of the most frequent consequences when working with biological material is acquiring an infection/infestation. History shows how workers working in the laboratory show a higher risk of infection than the rest of the population [144].

The parasites described earlier are considered Biological Agents (BA) that we can encounter in laboratories for research and diagnostics, creating occupational biological risk, since the lab activities involve constant contact with biological material. Although biological agents do not fit into strict categories, it is possible to assess the relative risk of a microorganism according to the classification in 4 risk groups (RG) drawn up by the WHO [145] and adopted by European Union with the Directive 2000/54/EC (Table 7):

- **RG1** (none or low individual or community risk): BA that is unlikely to cause disease;
- **RG2** (moderate individual or community risk): BA that can cause disease in human subjects, capable to constitute a risk to workers, but with low probability that it spread in the community; effective prophylactic or therapeutic measures are normally available;
- **RG3** (high individual risk, low community risk): BA capable of causing serious illness, represent a serious risk to workers, and can spread in the community, but effective prophylactic or therapeutic measures are available;
- **RG4** (high individual and community risk): BA that can cause serious illness and is a serious risk for workers, present a high risk of propagation in the community, effective prophylactic or therapeutic measures are not normally available.

All parasites considered in this review are included in the RG2 category, except for *E. granulosus, L. donovani* and *L. braziliensis*, included the RG3 category with double asterisk (**), which is capable of causing serious disease with no airborne transmission risks and limited chance of spread and for which there are usually effective preventive measures (EU Directive note 8, annex III) [146]. For this reason, the prophylactic measures are the same adopted for the BA of the RG2 group.

It is important to underline that this classification takes into account only the effects of BA on the immunocompetent worker, and not the possible effects

### Table 8. Containment measures against Laboratory Biological Agents extracted from Annex VI of Directive 2000/54/EC [146].

<table>
<thead>
<tr>
<th>Containment measures</th>
<th>BSL-2</th>
<th>BSL-3</th>
<th>BSL-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>The workplace is to be separated from any other activities in the same building</td>
<td>No</td>
<td>Recommended</td>
<td>Yes</td>
</tr>
<tr>
<td>The workplace is to be sealable to permit fumigation</td>
<td>No</td>
<td>Recommended</td>
<td>Yes</td>
</tr>
<tr>
<td>Infected material including any animal is to be handled in a safety cabinet or isolation or other suitable containment</td>
<td>Yes, where infection is by airborne route</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Input air and extract air to the workplace are to be filtered using (HEPA) or likewise</td>
<td>Yes, on extract air</td>
<td>Yes, on input and extract air</td>
<td>Yes</td>
</tr>
<tr>
<td>The workplace is to be maintained at an air pressure negative to atmosphere</td>
<td>No</td>
<td>Recommended</td>
<td>Yes</td>
</tr>
<tr>
<td>Surfaces impervious to water and easy to clean</td>
<td>Yes, for bench, floor and other surfaces determined by risk assessment</td>
<td>Yes, for bench, walls, floor and ceiling</td>
<td></td>
</tr>
<tr>
<td>Surfaces resistant to acids, alkalis, solvents, disinfectants</td>
<td>Recommended</td>
<td>Yes</td>
<td>Yes, via airlock</td>
</tr>
<tr>
<td>Access is to be restricted to nominated workers only</td>
<td>Recommended</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Efficient vector control, for example rodents and insects</td>
<td>Recommended</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Specified disinfection procedures</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Safe storage of a biological agent</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes, secure storage</td>
</tr>
<tr>
<td>Personnel should shower before leaving the contained area</td>
<td>No</td>
<td>Recommended</td>
<td>Recommended</td>
</tr>
<tr>
<td>Validated inactivation process for the safe disposal of animal carcases</td>
<td>Yes, on or off site</td>
<td>Yes, on site</td>
<td>Yes</td>
</tr>
<tr>
<td>A laboratory is to contain its own equipment</td>
<td>Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>An observation window, or, alternative, is to be present, so that occupants can be seen</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>
on the worker of risk categories, that is who has a higher probability of contracting a disease (or contracting a disease in a more serious form), following exposure of the pathogen, compared to the majority of the general population. Indeed, as reported in the WHO Biosafety Manual [145], referring only to risk groups is not sufficient for risk assessment. Other factors to consider are the natural routes of infection and other routes due to manipulation in the laboratory, the type of activity that is carried out in the laboratory (homogenization, sonication, centrifugation, aerosolization, etc.), the stability and resistance of the microorganism/pathogen in the environment, the concentration of the agent that is manipulated and the reports of previous laboratory-infections [147]. The prevention of biological risk is based on the adoption of technical, organizational and procedural measures, on the choice and correct use of appropriate PPE, the health education and health surveillance of workers.

Laboratories are distinguished as basic laboratories (Biosafety Level -1 and -2) and containment labs (Biosafety Level-3 and -4). The assignment of biosafety level takes into account structural features, available equipment, and activities performed. More information about Biosafety Levels (BSL) BSL-2 BSL-3 and BSL-4, listed in Annex VI of the Directive 2000/54/EC, is reported in Table 8.

The preventive measures taken in level 2 containment laboratories (Biosafety Levels-2 and Animal Biosafety Level-2) are recommended to be adopted in the research and diagnostic parasitology laboratories to achieve an acceptable level of safety [7]. It is absolutely recommended to use the universal standard procedures, which include hand washing, the use of gloves, lab coats, protective masks, goggles and visors, and other precautions to prevent accidental exposure. Therefore, the laboratory operator must use disposable gloves at each handling step of potentially infectious samples, before and during the sample preparation, wash the laboratory equipment, and wash the hands with water and neutral soap. It is mandatory to use lab coat with stretch sleeves to protect the wrists and wear clothes that can cover the legs. It is also recommended to wear face masks to reduce the possibility of transmission by air, as in the case of Cryptosporidium spp. or G. duodenalis [7]. Workers belonging to any risk category, such as immunocompromised and, in the case of T. gondii, pregnant women, must avoid working in workplaces with zoonotic risk [4]. In case of Leishmania spp. it is important to avoid accidental needlestick and also blood samples should be handled with care, even though fewer parasites generally are found in the bloodstream than in infected tissues.

Manipulation of biological agents requires identification of the best practices and integration of multiple strategies to control the possibility of spread of infections besides responding to unforeseen circumstances. In the parasitology laboratories, it is important to correctly perform the decontamination, disinfection and sterilization procedures, due to the ability of oocyst cysts and eggs to resist the external environment, resulting in all respects one of the major critical points for the prevention of any infestations [148].

Briefly, decontamination consists of cleaning of an instrument, device, or area with ordinary soap and water to primarily reduce the risk of infection. It is an essential pre-requisite to disinfection or sterilization processes to ensure the optimal activity of the disinfectants or sterilization processes. In fact, cleaning can be used to remove microorganisms and other associated contaminants (e.g., feces, blood, etc.) from a surface by physical means. Disinfection is generally a less-lethal process than sterilization; it eliminates nearly all recognized pathogenic microorganisms, but not necessarily all microbial forms. Disinfection does not ensure a kill level and lacks the margin of safety achieved by sterilization procedures [7]. Factors affecting disinfection are linked to physical and structural characteristics of several parasites, and for this reason, is essential to know the nature of the parasite which one can come into contact with but, above all, which chemical and or physical agent is most appropriate for its inactivation. Furthermore, is important to develop and test new disinfectant that could be effective on several parasitic species. In fact, due to the current pandemic situation caused by SARS-COV-2, the need of effective precautionary methods is increasing, leading to the development of new effective chemical solutions such as hydrogen peroxide combined with silver nanoparticles [155,156]. Other interventions could be the engineering and installation of self-disinfecting surfaces at the laboratories which could reduce the preliminary chances of contamination [157]. Additionally, plasma disinfection (ionized gas) could also be used to inactivate the pathogenic organisms in the parasitological examination facilities [158].

The moist heat used with autoclaves, with saturated steam under pressure, is the most reliable measure to sterilize the laboratory materials. Different heating cycles for varying time periods ensure sterilization [145] for example, 3 minutes at 134 °C, 10 minutes at
126 °C, 15 minutes at 121 °C and/or 25 minutes at 115 °C [7]

It is equally important to develop standard code of practice for handling infectious material to reduce the pathogen transmission. The disposable material must be correctly disposed in containers with the indication "hazardous medical waste at infectious risk". Sharp objects (slides, blades and syringes) should be disposed in rigid yellow containers (halibox) while, for the remaining disposable material, the container is made by disposable packaging, with an internal polyethylene bag inserted in a rigid and waterproof external container. In fact, waste of research on veterinary diagnostic activities is considered potentially hazardous at infectious risk. Carcasses and anatomical parts coming from the diagnostic activity of the Experimental Zooprophylactic Institutes, from the Departments of Veterinary Medicine, and from the Scientific Research Institutes and Centers are classified in the category according to Regulation (EC) No 1774/2002 [149]. These are disposed of in approved and certified incineration plants, or they are processed in an approved plant according to a specific method.

The main theme behind implementation of the control measures for the infectious agents is the protection of laboratory workers and general public through proper management (handling and disposal) of biological (infectious) wastes. A biological safety program, developed to minimize the risks associated with the handling and disposal of pathogenic organisms, is based on the transmission mode, pathogenicity of the organism and the susceptibility of the host. In the specific case of parasitic biological agents, it is important to evaluate for each parasite a method of decontamination, disinfection and possibly appropriate sterilization. In fact, from the document drawn up by the CDC (Centres for Disease Control and Prevention), Biosafety in Microbiological and Biomedical laboratories, emerges that zoonotic parasites have a grade 2 stability, for which zoonotic pathogens could be exposed to during the laboratory activities. A reduction in infection and infestation risks posed by the handling of parasites can be achieved through the systematic and timely application of a unified strategy. This includes the management of occupational exposures to various biological agents, the training of laboratory staff, and the implementation of standard cleaning and disinfection measures. A safety plan identifying potential biological hazards should be at the core of an effective management strategy to minimize accidental exposures. It is evident that for a greater understanding and analysis of the topic examined in the review, it is necessary to update data regularly on both epidemiology and environmental resistance and chemical agents of cysts / oocysts and eggs, which are reflected in the work environment, such as the laboratory (of parasitology), together with the development of new disinfectants, easier and safer to use.

Conclusions
The present review had the purpose of raising awareness among the operators of the diagnostic and research facilities who work in the field of parasitology, for educating them about safety by acquiring a greater consciousness of what kind of biological risks they could be exposed to during the laboratory activities. A reduction in infection and infestation risks posed by the handling of parasites can be achieved through the systematic and timely application of a unified strategy. This includes the management of occupational exposures to various biological agents, the training of laboratory staff, and the implementation of standard cleaning and disinfection measures. A safety plan identifying potential biological hazards should be at the core of an effective management strategy to minimize accidental exposures. It is evident that for a greater understanding and analysis of the topic examined in the review, it is necessary to update data regularly on both epidemiology and environmental resistance and chemical agents of cysts / oocysts and eggs, which are reflected in the work environment, such as the laboratory (of parasitology), together with the development of new disinfectants, easier and safer to use.

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