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REVIEW

Diagnosis of Pentastome Infections and the Need for Increased Awareness Among Medical Practitioners and Diagnosticians in the Developed World

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Abstract

Purpose of Review The patterns of movement of people, and animals, are changing due to climate change, immigration and ongoing refugee crises. Subsequently, many parasitic diseases, including pentastomiasis, are also changing their patterns of distribution. This review is aimed at raising awareness among medical practitioners and diagnosticians, especially in non-endemic areas for pentastomiasis, the disease, and highlighting the issues with the identification of pentastome infections in humans.

Recent Findings Pentastome infections in humans can be either visceral or nasopharyngeal in location. Visceral pentastomiasis is generally asymptomatic and is usually only discovered incidentally during examination for other issues or at autopsy. Nasopharyngeal pentastomiasis presents as an acute infection, generally following ingestion of raw or undercooked offal. Identification of the causative agent/s of pentastomiasis is based on general morphological features, determined through histology or radiology, with molecular confirmation of species rare.

Summary Although specific identity of the pentastome infection is probably not required from a medical viewpoint with regard to treatment of the infection, it is needed to improve our understanding of the epidemiology of infections. Additionally, with the increased movements of humans, and other animals, and their associated parasitic diseases, these parasites will start to appear in non-endemic areas. Medical practitioners and diagnosticians, as well as veterinarians, need to be aware of these parasites to ensure an accurate identification of the disease.

Keywords Pentastomiasis · Geographical distribution · Zoonotic disease · Diagnosis

Introduction

Climate change, immigration and ongoing refugee crises are changing the distribution of human parasites and their associated diseases around the world [1–3]. In addition, an increased level of interaction with wildlife through urban expansion and the need to ensure food security through an increased level of consumption of bushmeat or wild game increases risks of exposure to a variety of parasites and diseases [4]. Movements of animals, or even animal parts, either through accidental or deliberate means, can also change the risk of interaction with parasites and diseases [5]. Although some parasitic diseases, such as malaria, are well-known and easily diagnosed, many parasitic diseases, especially those predominant in wildlife, remain understudied, with limited knowledge of specific symptoms, effects and potential morbidity or mortality upon people. Thus, medical practitioners and diagnosticians must adapt to the potential of symptoms and pathologies associated with parasites and diseases new to their parts of the world [1, 2].

Pentastomiasis is one such disease that is prevalent in certain parts of the world, such as the Middle East, Africa and parts of south-east Asia [6, 7], but can move with people, remaining undiagnosed for a long time [2]. Additionally, the high number of asymptomatic cases or cases with general symptoms such as fever, nausea and abdominal pain prevents easy diagnosis [8, 9•, 10••, 11••]. Pentastomiasis is caused by infection with pentastomes, a group of obligate parasites, usually found as adults in the respiratory system

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of their vertebrate host [7]. The majority of the 124 accepted species [12] are found as adults in reptiles, although some are found in mammals [7]. As a group, pentastomes are a 'relatively neglected and poorly understood class of endoparasites' [7, p46].

Although a number of pentastomes have been identified infecting humans, the vast majority of cases belong to two species: *Linguatula serrata* and *Armillifer armillatus* [7, 13, 14]. However, the majority of 'identifications' of pentastomatid infections have occurred through a combination of radiology and histology, leading to a morphological-based species-level identification, although this is known to not be accurate, especially beyond the level of genus [7, 15]. Pentastomatids possess relatively few external characters upon which to base a species-level identification [7]. Few sclerotised structures, in combination with unknown levels of intraspecific variation in morphology and poor initial taxonomic descriptions, have resulted in a complicated taxonomy [7]. The utilisation of molecular techniques to verify the identification of a pentastomatid infection is crucial [15], although it is still rarely utilised [13, 14].

This review is aimed at highlighting the issues with identification of pentastome infections in humans through an examination of some cases in the literature. Additionally, the review wishes to raise awareness among medical practitioners and diagnosticians, especially in non-endemic areas for pentastomiasis, to the parasites as the potential for this disease to be presented to in these areas is increasing.

Background

Pentastomes are included within the Arthropoda [16] and possess an external cuticle, which is moulted between nymphal (larval) stages and chitinised hooks for attachment [7]. The adults are sexually dimorphic, with males generally much smaller and shorter-lived than females. Within natural infections of wild hosts, most are dominated by females [7]. Additionally, mixed species infections are common among natural wild hosts (Riley and Huchzermeyer [17], for example, found four species, across two genera, within the lungs of a single crocodile), and examination of each individual pentastome is required to ensure correct identification.

Subsequently, the taxonomy of pentastomatids is complicated and confusing. With relatively few external features on which to base an identification, 'soft' morphological characters, such as body length and number of annuli (external 'segmentation'), are generally used. Fixation and handling processes, however, are known to affect morphological dimensions of pentastomes, but due to the small numbers of individuals generally examined, levels of intraspecific variation are not known [7, 18]. The number of abdominal annuli is an important diagnostic character for some genera, including *Armillifer*, but there can be pronounced intraspecific variation within infections in a single host animal [7]. Only rarely is it possible to identify nymphs specifically, but for some genera, the definitive number of annuli has been acquired by the infective nymph, and it is possible to specifically identify at least nymphal porocephalids using this sole criterion [7].

Hook morphology and dimensions are useful taxonomic characters, due to their chitinous structure which is unaffected by fixation technique, and can reliably differentiate between genera [7]. However, the hooks should be removed from the pentastome and examined lying flat on a microscope slide to ensure accuracy of measurements [7]. It is important, also, to note that some pentastomes have markedly different morphology of hooks between developmental stages [19] and sexes [20] and that hooks increase in size between moults, so it is important to determine the stage and sex of the pentastome being measured [7]. In practice, hook data can only be meaningfully compared between fully adult specimens [7].

For many pentastomes, especially those collected from reptiles, species differentiation has been based on host species ecology [21]. The influence of environment and host on morphological variation of pentastomatids, however, is unknown, but increased research on pentastomatids in a particular geographical region or host group usually results in revised identifications of species [18]. Riley and Self [21], for example, found that a single, morphologically variable species, *Waddycephalus teretiusculus* infecting Australian venomous snakes, was actually 10 different species when examined in detail.

Most pentastome species have also been described prior to the advent of molecular techniques and require molecular characterisation and verification. However, it is vital that sequences are obtained from specimens that have accurate morphological characterisation to enable an accurate species identification [19, 20, 22–24]. Molecular identification of nymphs identified as *L. serrata*, for example, has shown high levels of variability between nymphs collected from different hosts and geographical areas [e.g., 25]. Additionally, none of the sequenced nymphs have provided a 100% match for the original *L. serrata* sequence, identified from a dog in Norway [25].

Compounding the taxonomic issues is the interchangeable use of common and scientific names of particular pentastomes. The Linguatulidae is a family name, containing the zoonotic genus *Linguatula*, but linguatulids are also the older alternate name for all members of the Pentastomida [7, 12] and is often used incorrectly, adding to the confusion [e.g., 27]. Additionally, the propensity for any specimen of *Linguatula* to be identified as *L. serrata*, often without morphological verification, increases the confusion, and recent molecular work has shown that there may be different species of *Linguatula* in areas traditionally thought to only contain *L. serrata* [10••, 20, 24]. And as often occurs in taxonomy, revisions change the names of parasites, moving them between genera and families, which is often not always immediately accepted, creating issues with synonyms and specimens being referred to by incorrect names. For example, *Armillifer moniliformis* has a long list of synonyms since its original description in 1836 [16], including the Australian subspecies *A. moniliformis australis*, first described by Heymons which was subsequently found to be a distinct species by Riley and Self [26].

Life Cycles

In general, life cycles for pentastomes involve sexually dioecious adults in the respiratory system of a vertebrate host [7]. Fertilised larvated eggs are released from the host, either through nasal discharge or in the faeces [7]. Eggs are ingested by intermediate hosts—dependent on the pentastome species, this can be an invertebrate insect host, a fish, a small lizard or mammal. Within the intermediate host, the egg hatches, releasing a first nymphal stage which undergoes a series of moults to become the infective nymph. During this process, the nymph may migrate through the body of the intermediate host, eventually encapsulating within mesenteries, organs or tissues. Ingestion of the intermediate host at this point releases the infective nymph which migrates to the respiratory system in the final host. Some species have a direct life cycle, but this appears limited to the genus *Reighardia* found in gulls [27] and potentially *Linguatula arctica* in ungulates [28].

Humans become infected with pentastomes through the ingestion of either eggs, leading to visceral pentastomiasis, or through ingestion of infective nymphs, leading to nasopharyngeal pentastomiasis, known as Halzoun or Marrara syndrome [7, 29]. Ingestion of eggs is usually accidental, with the food product contaminated by eggs from adult female pentastomes during the butchering process of the infected host (e.g., snake), or through ingestion of water contaminated by infected faeces [14]. Visceral pentastomiasis is generally asymptomatic, with diagnosis of infection occurring as incidental findings during surgery or autopsy [6, 11••, 14, 30]. Nasopharyngeal pentastomiasis, however, usually follows a meal containing raw or undercooked offal, with people presenting to medical attention within a few hours [7, 10••].

Pentastome nymphs appear to show active site selection in their hosts: *Linguatula* prefers the bronchial and mesenteric lymph nodes [31], while *A. armillatus* in man invades the liver, intestine and mesenteries [6, 14]. However, as with many aspects of pentastomes, further research is showing differences in localisation of nymphs in different types of intermediate hosts [32, 33]. Generally, nymphs are encysted in intermediate hosts within the last moulted cuticle inside a capsule of host origin and death of the intermediate host may be a cue for excystment [7]. Reports of freely migrating (excysted) *Linguatula* nymphs in intermediate hosts [7, 31–33] may reflect post-mortem migrations. If these do occur naturally, their significance is unclear [7].

Diagnosis of Infections in People

The pathology of pentastomatid infections in people is poorly understood. Human pentastomiasis varies enormously in its clinical and pathological manifestations. Pentastomiasis has been found to range from asymptomatic subclinical disease with minimal pathological reaction [9•, 14] to serious manifestations including abscesses and/or space-occupying lesions, or obstructions of vital passageways, and is commonly found in association with liver cancer [7, 34, 35].

The true extent of infection of people with visceral pentastomiasis is unknown [9•, 11••] as the majority of diagnoses of visceral pentastomiasis are made incidentally after a person has presented with generic symptoms (e.g., nausea and abdominal pain) or at autopsy [11••, 13, 14, 30]. This is thought to be related to a low overall parasite load [6] due to the accidental manner of ingestion of eggs, although there are some cases of visceral pentastomiasis that have been fatal [11••, 36, 37]. In areas where consumption of snake meat is common, however, incidence of infection can be high [36, 38], with almost 50% of Aboriginal Malays found to be infected with pentastomes at autopsy [30]. Infections can generally be linked back to consumption of food or water likely to have been contaminated with eggs from pentastomes, or through association with snakes (as in the case of a snake farmer; [39]), but there are sporadic cases where there is no obvious link (a French man who had worked with wood from Africa; [40]). Visceral pentastomiasis can be caused by any pentastomid species but is most commonly associated with *Armillifer* spp. [13, 14].

Infection with nasopharyngeal pentastomiasis generally results in acute, severe infections, following consumption of raw or undercooked offal, predominately from sheep, goats, cattle or camels [7, 10••]. Nasopharyngeal pentastomiasis is only caused by infection with *L. serrata*, and within endemic areas of the Middle East, symptoms are named based on the geographic area, being known as Halzoun in Lebanon and Marrara syndrome in the Sudan [7, 41]. Symptoms of infection are usually of short duration and restricted to the nasopharyngeal area with no systemic manifestations [7]. Similar to visceral pentastomiasis, the true extent of infection with nasopharyngeal pentastomiasis is unknown as many people in these endemic areas recognise the symptoms and know they will cease after a short duration and, subsequently, do not present to a medical practitioner [10••, 41]. Of infections with *L. serrata*, nasopharyngeal pentastomiasis was found to be the most common [10••].

Ocular pentastomiasis is where a pentastome nymph is located in the eye of the person [9•]. Thankfully, this appears to be a rare disease, with only 19 cases reported so far [9•], although many of the patients had subsequent permanent visual damage [9•, 38]. Nymphs of both *Armillifer* spp. and *L. serrata* have been recovered from affected eyes [9•, 34, 38, 42–46].

Histological, morphological or radiological examination are the principal methods of identification of a pentastome as the causative agent [8, 14]. Serological tests for pentastome infection, such as *Armillifer* spp., are rarely used in clinical practise [8] and have yet to be developed for detection of linguatualosis [7]. Molecular confirmation of identification of pentastome species found in people has only begun to be used recently [14, 47•], but is limited in certain parts of the world due to costs and access [14].

When whole specimens are recovered, as in the case of nasopharyngeal or ocular pentastomiasis, specimens are usually identified through morphology. Morphological examination of pentastomes generally involves the whole specimen being examined, and 'identification' made based on gross morphology of body shape (round (*Armillifer* or *Porocephalus* spp.) versus flat (*L. serrata*)) and number of annuli. Removal of hooks (as discussed above) and/or molecular confirmation of species identity is rare. In some reports, photographs of specimens are included, although misidentification of pentastomes still occurs. Wang et al. [48] reported a pentastome infection collected from a 3-year-old girl admitted to hospital with a 3-day fever and nymphs collected from discharged stool which were identified as *Armillifer agkistrodontis*, based on the number of annuli. Shao and Tang [49] reported a very similar pentastome infection (in a 3-year-old girl who presented to a hospital with a 3-day fever, abdominal pain, and anorexia with nymphs recovered from discharged stool) and with a very similar photograph. However, Shao and Tang [49] identified the nymphs as *L. serrata*, with no evidence provided and despite the earlier identification. Examination of the photograph indicates the infection to definitely not be *L. serrata*, based on body shape alone. In a separate case, Qiu and Jiang [50] reported that an infection previously identified as *Armillifer* sp. was, in fact, a nasal leech, showing that care needs to be taken in determining the identification of parasites.

As discussed above, taxonomy of pentastomids is often reliant on number of abdominal annuli [7, 51], a character which is not always easily determined in a histological section. Although species of *Armillifer* are capable of being differentiated based on annulus count, without complete specimens, only limited conclusions regarding even genus-level identification should be made [47•]. In many histology cases, the pentastomid has degenerated to such an extent that only sections of the cuticle remain. However, despite this, a diagnosis of *Linguatula*, and more specifically *L. serrata*, has still been made by the fact that the cuticle possessed external spines [52, 53], although nymphs of other species of pentastomes also have annular spines [19] as the spines are used by the nymph during migration through the body of the intermediate host [7].

The use of radiological techniques is often used for diagnosis of visceral pentastomiasis, with nymphs found mainly on the liver, intestinal wall or within the mesenteries of the abdominal cavity [11••, 14]. Nymphs can be visualised as conspicuously annulated structures, but older infections of *Armillifer* spp. generally calcify into a characteristic C-shape [11••, 14]. Often radiology will only identify calcified cysts in chronic infections [11••].

In addition to the physical identification of a pentastome specimen, information should be gathered from the patient with regard to dietary and religious/traditional customs, especially where snakes may be a totem, as well as history of travel [1, 5, 10••, 14, 38, 54]. Examination of snake meat sold in local markets in the Congo Basin showed a high incidence of *Armillifer* [38]. Infection was likely to pass to people due to consumption of uncooked meat or indirectly through contaminated hands, kitchen tools or washing water used in butchering of snakes [11••, 38]. *Armillifer* infections were found to be more prevalent in men [11••], whereas *L. serrata* was often found in women involved in food preparation [10••].

Molecular confirmation of identification of pentastome species found in people has only begun to be used recently [11••]. Tappe et al. [55] and Tappe et al. [47•] sequenced pentastome specimens collected from cases in the Democratic Republic of the Congo, finding a mixture of species of *Armillifer*, with one infection also containing a *Raillietiella* sp. [47•]. The majority of these cases were also examined and identified histologically. One example of molecular verification of *L. serrata* was that of a nymph collected from the eye of a child in Austria [44]. However, issues with variability between types of genes sequenced are a problem [20, 23, 24]. The Austrian sequence generated was for 18S rRNA [44] which did not match the sequences for the identified *L. serrata* collected from a dog [25]. It has been suggested that Cox1 and 28S rRNA are potentially better at differentiating species, at least for *Linguatula* [23].

Identification of pentastomiasis needs to occur through the finding of either an entire nymph or identifiable fragments of degenerated nymphs, but should be limited to a diagnosis of pentastomatid, if there is no molecular confirmation [29]. This is especially true for areas of the world where diagnosis of pentastomiasis is rare and misdiagnosis may occur [10••, 29]. Most reports of infection with

Linguatula are identified as *L. serrata* [10••, 56], although recent molecular work has shown that there may be different species [10••]. Additionally, a range of other pentastome species have been recovered from people: *Leiperia cincinnalis* (also called *Pentastome gracile*; [34, 57]), *Raillietiella hemidactyli* [58], *Sebekia* sp. [59], *Porocephalus crotali* [58], and *Porocephalus taiwana* [48]. Increased use of molecular techniques should assist with identification of the various pentastomes found to infect people across the world.

The Need for Increased Awareness

Many immigrants and refugees have undiagnosed or poorly managed medical conditions, often due to a breakdown of the health system of their home countries during economic and social unrest [1, 3]. As industrialised/developed countries receive increasing numbers of these immigrants and refugees, especially from parts of the world where pentastomiasis is endemic, this disease will become more frequently encountered by medical practitioners and diagnosticians [2, 13, 60] (see Table 1). Infections with *L. serrata*, *A. armillatus* or an *Armillifer* sp. have already been diagnosed in Europe and Northern America (Canada and the United States) as well as in South Africa. The *Armillifer* spp. infections have all originated in western or central African countries (Table 1). For the infections of *L. serrata*, however, only one has come from the traditional endemic area of the Middle East, with the other infections originating from countries (Russia, Hungary and Kazakhstan) (Table 1) which are not considered traditional endemic countries, highlighting the many issues with determining the true distribution of *L. serrata*.

Pentastomes are often not mentioned in medical parasitology textbooks, so diagnosticians may not easily diagnose an infection [2]. Additionally, although pentastomes may have been prevalent in some of these countries, such as the United Kingdom, last century, they have subsequently disappeared and may not be initially recognised as a potential disease issue [61].

Raising awareness among medical practitioners of the most common and serious medical conditions that occur in immigrant groups from different geographic regions is important for early diagnosis and treatment of diseases that may initially be asymptomatic [1, 62]. Additionally, culinary habits of countries change with the arrival of immigrants [63–66], leading to potential increases in infections with parasites in some groups. Barton et al. [65] discussed the potential zoonotic risks of consuming feral animals in Australia especially given recent research which had reported *L. serrata* in rabbits [33], wallabies [32] and goats [67].

In addition to the movement of people, there has been an increase in the rehoming of street dogs across the world [5, 68]. Infections with *L. serrata* in these dogs have been reported and veterinarians, potential pet owners as well as medical practitioners, need to be aware of the risk of spreading zoonotic pathogens through importing/exporting dogs and other animals [5, 69]. In some areas of the world, reptiles are also popular pets, and although unlikely to be consumed, the risks of potential zoonotic parasites, especially if the reptile has been wild-caught, need to be identified.

Conclusions

Although specific identity of the pentastome infection is probably not required from a medical viewpoint with regard to treatment of the infection, it is needed to improve our understanding of the epidemiology of infections, including full host ranges as well as potential ways of preventing infections. As patterns of human movement and interactions with wildlife increase and change, due to climate change and various socio-economic factors, common infections in certain areas of the world will become more prevalent in new, most likely developed, countries. As such, veterinarians, medical practitioners and diagnostic technicians need to be aware of the possibility of these infections.

Despite the variety of species within other pentastomatid genera and the ability to differentiate species of *Armillifer* based on geographic distribution, there is almost a blind spot when it comes to identification of *Linguatula*. *Linguatula serrata* is the default diagnosis of every 'tongue worm' infection, anywhere in the world, even in regions where other species of *Linguatula* have been described. Although *L. serrata* may be the only species within the genus that infects people, no one appears to have even considered the possibility that another species of *Linguatula* could be involved as a zoonotic parasitic infection, especially as a nymphal stage that accidentally finds itself in the wrong host. Further research is required to correctly identify pentastomids found infecting people to ensure accurate diagnoses.

Table 1 Cases of human pentastomiasis diagnosed in countries other than the original source of infection. MLN, mesenteric lymph nodes; NS, not stated

Case	Country of diagnosis	Pentastome species	Location in body	Age	Sex	Reason for presentation	Diagnosis method	Country of origin	Reference
1	USA	*L. serrata*	Liver, MLN, abdominal cavity	4	M	Presented other symptoms	Histology	Middle East	[70]
2	France	*Armillifer* sp.	NS	NS	NS	NS	Radiology	Africa	Piéron et al. (1982) in [14]
3	Canada	*L. serrata*	MLN	47	M	Not stated	Histology	Hungary	[71]
4	USA	*A. armillatus*	Abdominal cavity	35	M	Presented other symptoms	Radiology	Liberia	[72]
5	Canada	*A. armillatus*	Liver, abdominal cavity, thoracic cavity	28	M	Autopsy	Histology, gross morphology	Nigeria	[73]
6	France	*Armillifer* sp.	Abdominal cavity	50	M	Presented other symptoms	Radiology	Gabon	[74]
7	France	*A. armillatus*	Liver, abdominal cavity, thoracic cavity	26	M	Autopsy	Gross morphology	Democratic Republic of Congo	[37]
8	France	*A. armillatus*	NS	34	F	NS	Radiology	Cameroon	Touze et al. (2001) in [14]
9	France	*A. armillatus*	Liver, abdominal cavity, thoracic cavity	40	M	Presented other symptoms	Radiology	Mali	[75]
10	Spain	*Armillifer* sp.	NS	25	F	NS	Laparoscopy	Nigeria	Martin-Rabadan et al. (2005) in [14]
11	Germany	*L. serrata*	Thoracic cavity	39	M	Presented other symptoms	Radiology, histology	Russia	[76]
12	South Africa	*A. armillatus*	Liver	39	M	Presented other symptoms	Radiology	Nigeria	[77]
13	Germany	*L. serrata*	Thoracic cavity	NS	NS	Presented other symptoms	Radiology, histology	Kazakhstan	[13]
14	Germany	*A. armillatus*	Liver	23	M	Autopsy	Molecular, histology	Togo	[78]
15	The Netherlands	*A. armillatus*	Abdominal cavity	23	F	Presented other symptoms	Gross morphology	Liberia	[60]
16	Italy	*A. armillatus*	Liver	23	M	Autopsy	Histology	Nigeria	[79]
17	France	*Armillifer* sp.	Liver	16	F	Presented other symptoms	Radiology	Cameroon	[80]
18	France	*Armillifer* sp.	Abdominal cavity	40	M	Presented other symptoms	Radiology	Central African Republic	[80]
19	France	*Armillifer* sp.	Liver, thoracic cavity	31	M	Presented other symptoms	Radiology	Democratic Republic of Congo	[81]
20	France	*Armillifer* sp.	Abdominal cavity	21	M	Presented other symptoms	Radiology, histology	Guinea	[82]
21	USA	*Armillifer* sp.	Liver, abdominal cavity, thoracic cavity	61	1	Presented other symptoms	Radiology	Togo	[83]

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Competing interests Shokoofeh Shamsi is on the Editorial Board for Current Clinical Microbiology Reports. Diane P. Barton is on the Editorial Board of another Springer journal, Systematic Parasitology

Conflict of Interest Prof. Shamsi is the Parasitology Section Editor for the Journal. Dr. Barton has nothing to disclose.

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