CHAPTER II

LITERATURE REVIEW

2.1 Morphology of *Alaria alata*

Alaria alata has first been described by Goeze in 1782, and is presently classified as below:

Class:	flukes (trematodes)
Subclass:	Digenea
Order:	Strigeatida
Family:	Diplostomatidae
Species:	*Alaria*
Type:	*Alaria alata*
Scientific name:	*Alaria alata*

According to Pearson (1956), the adult stage of *Alaria* spp. is characterized as follows. The *Alaria* spp. size is about 3–6×1–2 mm. The body is clearly separated into two parts. The anterior end involves four clavate cells within the oral sucker (0.06–0.07×0.06–0.08 mm) (Pearson, 1965). In this part, some glandular units were observed but their ducts were not espied. The posterior end is short and cylindric with a typical short intestine. The muscular ventral sucker is approximately 0.04–0.1×0.04–0.09 mm. The ventral sucker is operated for digestion and absorption of mucus and tissue from the wall of the host intestine. More details are described in Figure 3.

According to Hiepe (1985) and Lucius *et al.* (1988), the dimensions of *Alaria* spp. eggs are 110–140×70–80 μm. Development from egg to an individual adult takes about 92–114 days (Buller, 2012).

The metacercarial stage of *Alaria* spp. is oval in shape, a thin-walled, almost transparent vesicle of 0.4–0.7 mm length and 0.2 mm breadth with fine parallel lines on it (Buller, 2012). In these cysts, one could identify the whitish larva with a magnifying glass.

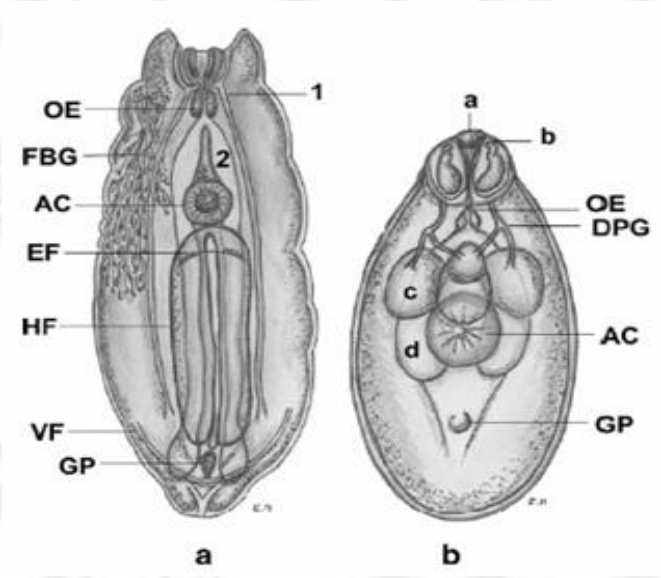

Figure 3 Fully developed adult (a) and mesocercarial (b) stage of *Alaria* spp.

1. Remnant of penetration gland duct; 2. Caecum (elonged);

a: oral opening and oral opening and oral sucker, b: gland cells,

c: penetration glands, d: caecum,

OE: oesophagus, FBG: fore body glands and their ducts, AC: acetabulum, EF: edge of fold over anterior and of holdfast organ, HF: holdfast organ, VF: ventral lip of spathiform fore body, GP: genital primordium. DPG : duct of penetration gland

Source: Möhl et al., 2009

2.2 Life cycle

The adult forms of *Alaria alata* settle in the intestines of (wild) carnivores, i.e. definite hosts. Eggs are shed via faeces into fresh-water sources such as ponds, plashes, etc. and hatch into miracidia. The miracidia penetrate the freshwater snails from the genus *Planorbis* such as *Planorbis planorbis* and *Anisus vortex* as first intermediate hosts of the flatworm (Murphy *et al.*, 2012). The miracidium migrates and matures in the snails as a sporocyst, an asexual form. The sporocysts develop into cercariae in the water, a free-living stage (Castro *et al.*, 2009). The cercariae attach and infect an amphibian such as tadpole, frog or toad which is considered as second intermediate hosts of the parasite. The cercariae develop inside the second intermediate hosts into mesocercariae, a sexually immature larval stage, that have an affinity to move freely in the host muscle tissues. When the infected second intermediate host is eaten by a definitive host, the mesocercariae migrate from the intestinal tract to the lungs, and develop into metacercariae. These metacercariae migrate via trachea and pharynx to the intestinal tract where they develop into the mature stage. The amphibian may not be digested by a definite host but by paratenic hosts (a host that does not experience pathology from infection and inside which a parasite does not mature and maintains the form of mesocercarial stage) such as small mammals, reptiles (snakes), and birds (Castro *et al.*, 2009, Johnson, 1979, Wolfe *et al.*, 2001). Such paratenic hosts can amass many mesocercariae, and only when the paratenic host is devoured by a definitive host, the life cycle is completed (Johnson, 1979), see Figure 4.

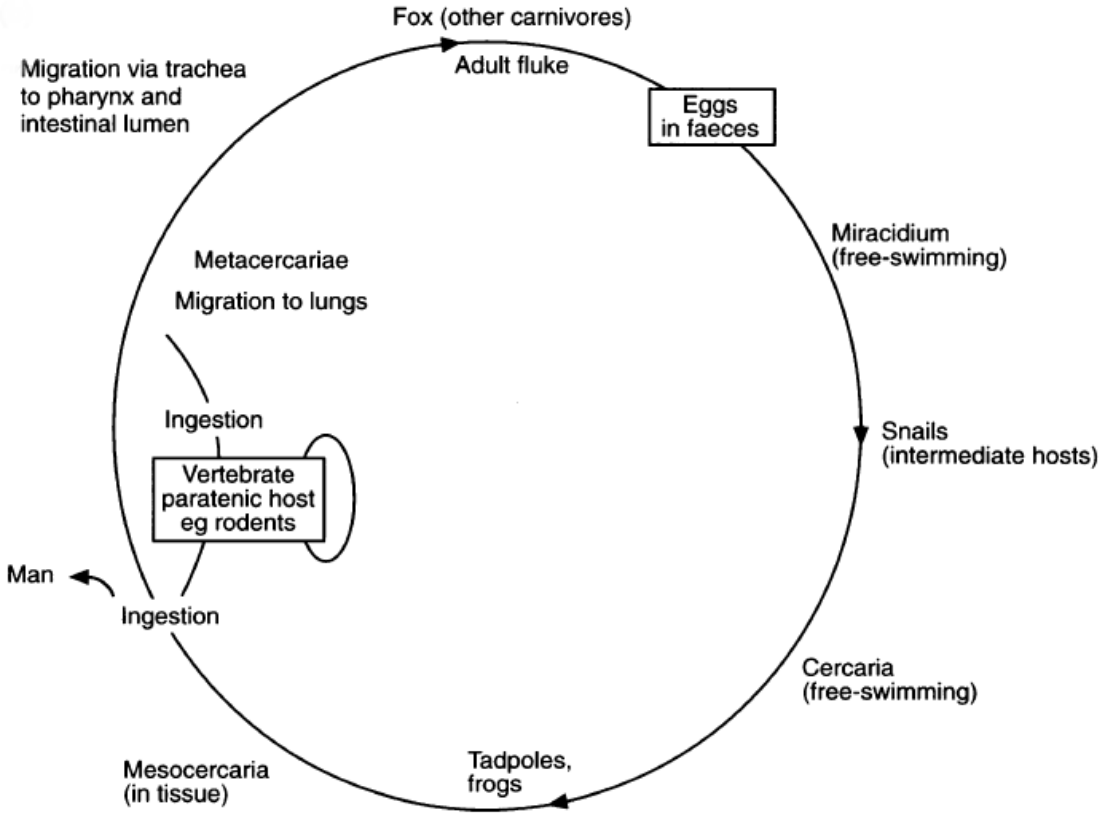

Figure 4 Life cycle of *Alaria* species: developmental stages
Source: Wofte et al.,2001

Alaria species are distinguished by morphological differences, so there is overlap of hosts among *Alaria* species. Small and large carnivorous mammals ranging from canids (foxes, coyotes, and wolves) to mustelines (badgers, skunks, and minks) and even domestic dogs and cats, are the definitive hosts (Figure 5) (Bruzinskaite-Schmidhalter *et al.*, 2011, Foster *et al.*, 2009, Castro *et al.*, 2009). In a research of Fischthal and Martin in 1977, *Alaria* was also found in a mountain lion in Paraguay (Fischthal and Martin, 1977). There is apparent overlap among paratenic and definitive hosts for *Alaria* species and even within species of *Alaria*, in that the age of a host can determine whether the host is definitive or paratenic. Shoop and Corkum in 1983 and 1984), discovered that a paratenic host could transmit mesocercariae vertically via blood circulation or through breast milk in pregnant feline and through

mammary glands of lactating mice (Shoop and Corkum, 1984, Shoop and Corkum, 1983b, Shoop and Corkum, 1983a).

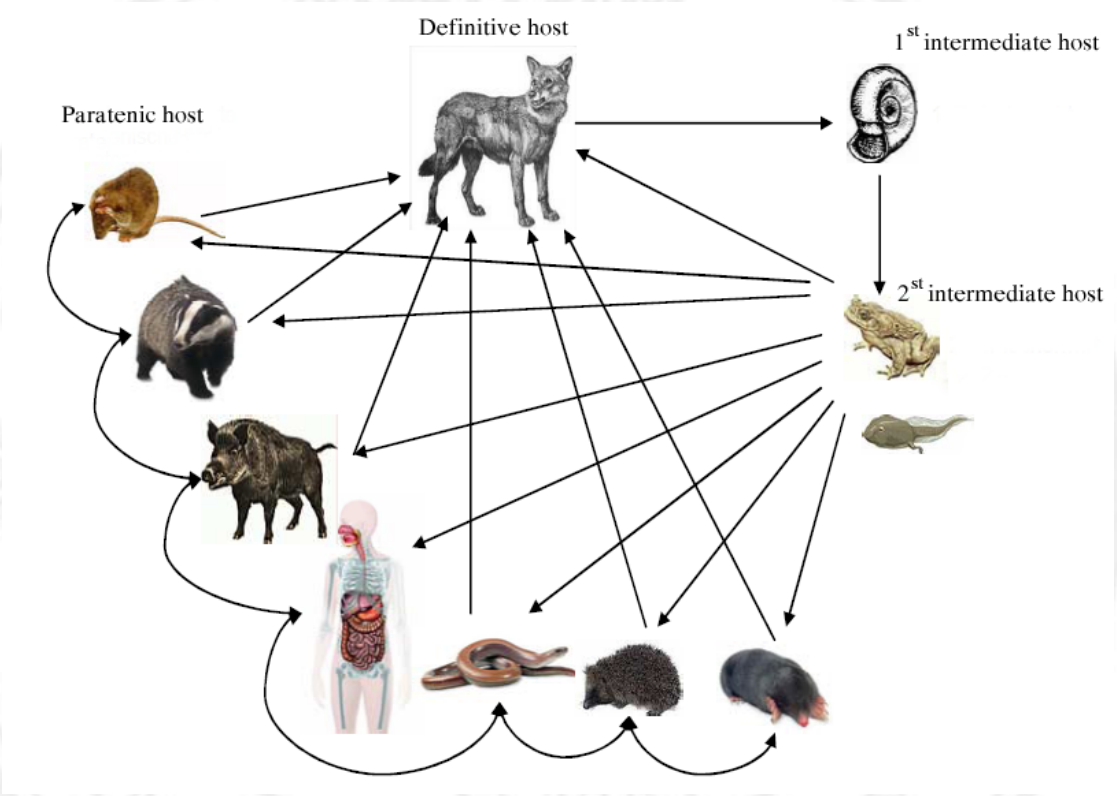

Figure 5 Possible relationship between the hosts of various stages of *Alaria* spp.
Source: Lücker et al., 2010

2.3 Pathogenesis and clinical symptoms referring to Alariosis in human

Alaria alata mesocercariae were detected during official *Trichinella* inspection accidentally. Only recently, initiatives were taken to conduct a reassessment of the potential human health risk by this parasite.

The pathogenicity of the adult form of *A. alata* is regarded as low. This might be due to the low level of awareness on this zoonosis and the poor detectability of mesocercariae as well as a high number of unreported cases. Odening (1961b) proved that *Alaria* mesocercariae can infect to primates, a paratenic host closely related to

humans, with severe damages. The present review of the literature on *Alaria* biology shows that the human exposition risk should not be considered negligible because a general lack of knowledge in *Alaria* biology can confound any risk analysis. Overall pathogenicity is associated to high infestation densities and repeated rather than single infections. Since 1973, several reports about human larval alariosis have been published as summarized in Table 1.

Table 1 Reported cases of human larval alariosis

Year	Parasite	Location	N	Manifestation	Infestation route and vector	Author
1969	*Alaria* (?) mesocercariae (?)	CA, USA	1	Eye	(?), (?)	Byers and Kimura 1974, McDonald et al., 1994
1972	*Alaria* mesocercariae	Ontario, Canada	1	Eye	Smear infection with the preparation of frog legs	Shea et al., 1973
1975	*Alaria Americana* mesocercariae	Ontario, Canada	1	Generalized (see Table 3), lethal	NU (frog legs)	Freeman et al., 1976, Fernandez et al., 1976
1975	*Alaria* mesocercariae	LA, USA	1	Skin	NU (venison, raccoon (?))	Beaver et al., 1977
1988	*Alaria* mesocercariae	CA, USA	1	Eye	NU (venison) or frog legs (PSI)	McDonald et al., 1994
1990	*Alaria Americana* mesocercariae	CA, USA	1	Eye	NU (venison) or frog legs (PSI)	McDonald et al., 1994
1993	*Alaria Americana* mesocercariae	Manitoba, Canada	1	Respiratory tract, skin	NU (wild goose (?))	Kramer et al., 1996

N: cases; (?): unconfirmed, unknown; PSI: possible smear infection; NU: nutritional

Source: Möhl et al., 2009

The orbit ranges from low-grade symptoms (respiratory organs, skin) over serious diseases of the eyes to one death after apparently receiving a massive dose of infectious agent. Implied vectors are especially frog legs but also meat of wild geese. In all cases it is assumed that no sufficient inactivation by heating took place.

In a research in 1976, Freeman reports on symptoms of human alariosis (Freeman *et al.,* 1976). It implicates various clinical signs such as low-grade respiratory, cutaneous symptoms, or diffuse unilateral subacute neuroretinitis (DUSN), or anaphylactic shock with lethal consequence. Löscher and Sonnenburg, (2005) mentioned that an important issue is that almost all trematode infections are related to eosinophilia and increase in IgE (Möhl *et al.*, 2009). It means that a general

anaphylactic reaction may arise from the infected agent by repetitive oral intake. Bork (1985) and Egger (2005) described the symptoms of an anaphylactic shock range from tachycardia and drop in blood pressure to vasomotor collapse and unconsciousness (Möhl *et al.*, 2009). Therefore, *Alaria alata* as a potential parasite of man in the state of mesocercariae, should receive attention as zoonotic agent.

2.4 A special lethal alariosis case in human

In a Canadian case described by Freeman *et al.* (1976), the patient complained about tightness in the chest and abdominal symptoms after several long hikes. Within 2 days of the initial illness, the patient developed flu-like symptoms like head- aches, fever, faintness, and cough, and on the third day, showing severe dyspnea and hemoptysis. On the fourth day, the patient became comatose, and skin petechiae were evident. The tentative diagnosis was viral pneumonia, and he was treated with broad-spectrum antibiotics. After the treatment failed, biopsy of a skin lesion and an open-lung biopsy were performed. The tissue sections of fixed lung tissue contained lengthwise sections of a fluke which was tentatively identified as *Alaria* spp. mesocercaria. By the ninth day, after initial symptoms, the patient died in the hospital. At autopsy, practically all viscera showed extensive local or diffuse hemorrhage. Several thousand mesocercariae were estimated to have been present within the viscera and nearly all organs (Freeman *et al.*, 1976). The cause of death was asphyxiation from extensive pulmonary hemorrhage, probably due to immune-mediated mechanisms, after repetitive oral intake of *Alaria americana* mesocercariae. The possibility that the infective dose of mesocercariae might have been ingested with drinking water was investigated and ruled out. The authors concluded that the victim ate uncooked or more likely inadequately cooked frog legs heavily infected with mesocercariae.

2.5 The epidemiology of *Alaria* spp. in the world

A flatworm parasite of the genus *Alaria* was generally detected throughout North America, South America, and Europe (Möhl *et al.*, 2009). Eight species of

Alaria were discovered in North America: (1) *Alaria americana*, (2) *A. arisaemoides*, (3) *A. canis*, (4) *A. intermedia*, (5) *A. marcianae*, (6) *A. mustelae*, (7) *A. nasuae*, and (8) *A. orgeonensis* (Buller, 2012). Species differentiation of *Alaria* is highly subordinate to morphological characteristics (size and position of organs) of adult parasites (Riehn *et al.*, 2010). On the other hand, showed that *A. americana, A. canis, A. marcianae,* and *A. mustelae* to be equivalent since they adapt to similar ecology (hosts and geographic region) (Buller, 2012).

There are only a few surveys of *Alaria* prevalence nowadays. Surveys of paratenic and second intermediate hosts report prevalence ranging from 3-5% in raccoons in Louisiana, Florida (Shoop and Corkum, 1981).

Hiepe (1985) pointed out that *Alaria alata* in Central Europe was rarely detected in domestic animals, and little attention was paid (Möhl *et al.*, 2009). In wild animals, however, the parasite is found regularly. However, adult *Alaria marcianae* form was reported in domestic cats (Shoop and Corkum, 1983b, Shoop and Corkum, 1983a) and adult form of *Alaria alata* in domestic cats in Uruguay (Castro *et al.*, 2009).

Duscher (2011) reported that the *A. alata* prevalences in red foxes in Austria ranged between 0.16 and 22.06 % in different areas of the country with higher prevalence found in the eastern parts of Austria (Duscher, 2011).

In a wildlife survey for zoonotic diseases in 2009 and 2010, prevalence of adult *A. alata* was 21 to 26% in Europe (Murphy *et al.*, 2012).

In summary, there were few studies on *Alaria* spp. and their biological data seems complex. A larger range of animals, e.g birds, carnivores, reptiles and amphibians were reported as the hosts in developmental stages of the trematode. Most studies on *Alaria* spp. were conducted in Europe and America, which was of limited use when the whole picture of *Alaria* spp. in the world has to be assessed. *Alaria* spp.

data in Asia and other continents are lacking. Therefore, the global *Alaria alata* prevalence is unknown now and the risk level is not yet assessed properly.

2.6 Diagnostic measure in animals

2.6.1 *Alaria* spp. egg detection method

Like other trematodes, eggs of *Alaria* spp. have some characters such as big shape and heavy weight. Therefore, the method can be used to detect the eggs of *Alaria* spp. should be a sedimentation technique. The process of this technique involves homogenizing feces samples with sterilized water and allowing time for solids including, if present, *Alaria* spp. eggs to settle at the bottom. The supernatant fluid is then discarded and repeated the process several times until the sediment is clear. The sediment is examined under microscope or stereoscope for presence of *Alaria* spp. eggs.

2.6.2 History of *Alaria* mesocercariae Migration technique

Although the detection of adult *A. alata* is well standardized, a specific detection method for *A. alata* mesocercariae in meat has been developed only quite recently. In the beginning, Hemmert-Halswick and Bugge (1934) pointed out that encysted larvae only occur on muscle surfaces (Riehn *et al.*, 2010). Mesocercariae, which are located within the muscular tissue, show no surrounding cyst or capsule. The *Trichinella* compression method was applied for detecting *A. alata* mesocercariae. Trichinoscopy was used to *Trichinella* detection until the pooled digestion method was implemented in the 1970s. Although the pooled digestion method is replacing trichinoscopy, the compression method has some advantages for the detection of *A. alata* mesocercariae. The *A.alata* mesocercariae detection by both methods mentioned above gave discrepant results. A result for testing *A. alata* by the compression method from researches of Marinculic (2007) and Pozio (2010) is 3 of 210 tested wild boars positive in Croatia. In contrast, other studies showed up to 91% of wild boars from Croatia to be positive using the digestion method.

The vastly differing biology of *Trichinella* and *A. alata* suggests that the official digestion methods, *Trichinella* inspection method (TIM), for *Trichinella* spp. might be unfit for *A. alata* mesocercariae detection or, at least, be the cause of false-negative results on a so far undefined level (Möhl *et al.*, 2009). According to Große and Wüste (2006), this hypothesis is extremely countenanced by official *Trichinella* inspection since this method was unable to find *A. alata* mesocercariae in any sample of the individual animals after positive results had been obtained in the respective pooled samples (Portier *et al.*, 2011).

Based on the observations that the parasite (1) shows a high affinity to liquids and (2) moves actively out of the tissues, a larvae migration technique was modified by Riehn *et al* (2010) to *Alaria* mesocercariae , *Alaria* mesocercariae Migration Technique (AMT).

For method comparison, one hundred eighty-six tissue samples of 18 wild boars were tested by both TIM and AMT in parallel by the above-mentioned authors. The results are presented in Table 2.

Table 2 TIM efficiency/quality as judged by AMT

	AMT +	AMT -	Total
TIM +	27	0	27
TIM -	38	121	159
Total	65	121	186

TIM: *Trichinella* inspection method

AMT : *Alaria* mesocercariae migration on technique

Source: Riehn et al., 2010

A first application of the newly developed *A. alata* mesocercariae migration technique demonstrated that the detection of positive samples of *A. alata* mesocercariae was improved in comparison with HCl - pepsin digestion, independent on the type of tissue (muscular, adipose, and/or connective tissue) used. A direct comparison between AMT and TIM proved that the sensitivity of AMT to detect *A. alata* mesocercariae in tissues of wild boars is about 58% higher than that of TIM. In my study, the samples will be tested by AMT as described by Riehn *et al.* (2010).

2.6.3 PCR approach for differentiation of *Alaria* spp. mesocercariae

Riehn *et al.* (2011) were successful to introduce a PCR technique to distinguish between *A. alata* mesocercariae with other *Alaria* spp. mesocercariae based on external characteristics and comparative morphology of adult flukes (Riehn *et al.,* 2011). The primer pair was selected to amplify a 303-bp region of the *A. alata* genome. The primer pair was set up following

DME-F: 5`- CTTAGCTGCGGGTTCCTGCT -3` and

DME-R: 5`-CTTAGCTGCGGGTTCCTGCT -3`

This molecular method helps to identify *A.alata* mesocercariae quickly and can be very useful for diagnostic and epidemiological purposes (Riehn *et al.,* 2011).

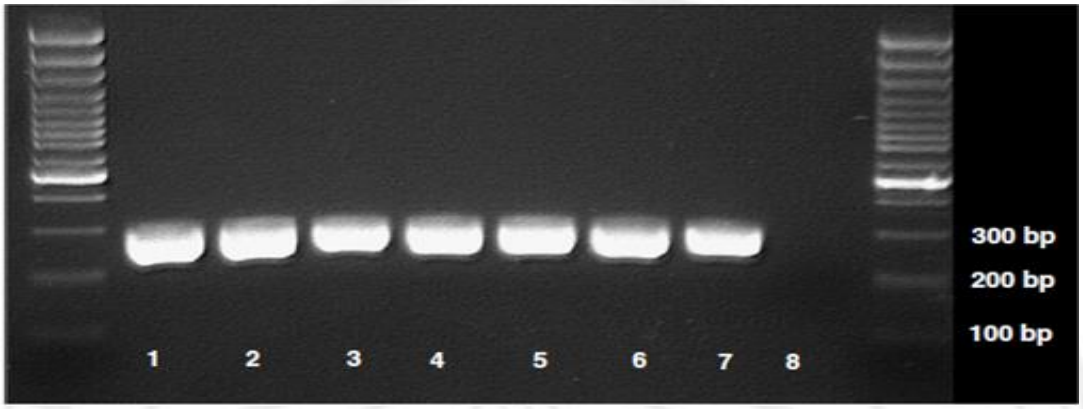

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Figure 6 PCR products by use of specific oligonucleotide primers DME-F and DME-R

Lanes 1-6: Alaria spp. specimens selected by means of morphological and morphomeric criteria; lane 7: positive control; lane 8: negative control: water

Source: Riehn et al., 2011

2.6.4 Serological test for *Alaria* spp. antibody detection

Currently, serological techniques for detecting antibodies against *Alaria* spp., in particular *A. alata*, are not available. This complicates distinguishing infected paratenic hosts among the population in vivo.

2.7 Awareness about *Alaria alata* in public

Since the prevalence of *Alaria alata* in animals is known only in certain regions and the incidence of human alariosis is virtually unknown, public health authorities can not issue any comment to public. For risk management, the development of suitable methods for detecting *Alaria alata* in definite as well as paratenic hosts and in humans should be set up, and examinations of the actual prevalence, disease severity and distribution should be conducted. In brief, there were few studies on *Alaria* spp. and its biological data seems to quite complex. A larger number of vertebrate animals, e.g. birds, carnivores, reptiles and amphibians can act as the hosts of the parasite. Lack of data, shortcomings in detection methods and low

awareness in the public to this potential zoonosis, may lead to some problems caused by *Alaria alata* in future.