



Putting the Spotlight on Opisthorchiasis: The Dread of the Western Siberian Region

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Review Article

Volume 4 Issue 1

Received Date: February 17, 2020

Published Date: March 10, 2020

DOI: 10.23880/phoa-16000151

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Abstract

Introduction: Opisthorchiasis is no doubt one of the most neglected infectious disease inspite of its huge medical importance in some parts of the World. The past decade have seen a resurgence of interests in research relating to this public health issue, however there is still a lot to be done.

Social Model: Not many models have been explored in Western Siberia to deal with the opisthorchiasis epidemic when compared to the different models that have been used for other regions affected by similar disease.

Life Cycle: The complex life cycle of *Opisthorchis felineus* has humans and other feline species as definitive host and is really prevalent among the aboriginal population of the Western Siberian because of their habit of eating raw or undercooked fresh water fish (Cyprinidae) which are intermediate host of the parasite.

Diagnosis and Treatment: Diagnosis involve the use of stool microscopy, other methods such as mAb ELISA, LAMP and so on are used, while the common treatment is the use of 25-40mg of the antihelminthic drug_Praziquantel.

Conclusion: The spotlight must be put on *O. Felineus*.

Keywords: Opisthorchiasis; Life Cycle; Cyprinidae; Western Siberian

Introduction

The opisthorchiidae family of the trematode class is of high economic, veterinary and medical importance, with over 45 million infected people from all round the globe [1,2]. *Opisthorchis felineus*, *Opisthorchis viverrini*, and *Clonorchis sinensis*, three of which belong to the aforementioned family though occasionally overlapping in terms of geographical distribution have a distinct pattern: *O. felineus* is endemic in Europe and Russia particularly the arctic region of Western Siberia; *C. sinensis* in China, the Republic of Korea, and northern Vietnam; and *O. viverrini* in Southeast Asia [1,3].

A general feature of all Opisthorchiidae is their high specificity to the first intermediate host. Typically, only one or several closely related snail species act as the first intermediate host for Opisthorchiidae, whereas the lists of second intermediate and definitive hosts may comprise up to several tens of species [4].

A study by Fedorova *et al* showed that in the whole of Russian Federation in 2011 to 2013, the average annual incidence of *O. felineus* infection was between the range of 15.7 to 33.7 cases per 100,000 population [5]. However, the major habitat of *O. felineus* in Russia is the Ob-Irtysh river

basin, which spans the south to north in the Western Siberia region. Compare to the European part of Russia and Eastern Siberia, the prevalence of *O. felineus* infection in Western Siberian people has been estimated to be up to 80%, about 100 to 1000 times more [5] In these regions, the prevalence rates of opisthorchiasis exceed 100 per 100 000 people [6]: Khanty-Mansiiskiy Autonomous Region (600 per 100 000 people), Tyumen Region (201 per 100 000 people), Tomsk Region (189 per 100 000 people), and Novosibirsk Region (127 per 100 000 people) [6].

Social Dynamics Model of Western Siberian Aboriginal population on Opisthorchiasis

The social dynamics model of *O. felineus* transmission in Western Siberia was designed for preparing and developing an integrated opisthorchiasis control programme in the region [5]. The model attests to the specificity of transmission dynamics in Western Siberia instead of using the one size fits all approach. The model also underpins the importance of understanding this public health issue as embedded in social networks and animated by a variety of locally-specific linkages between ways of life, food and health cultures, health-care systems, economies, and landscapes [5,6].

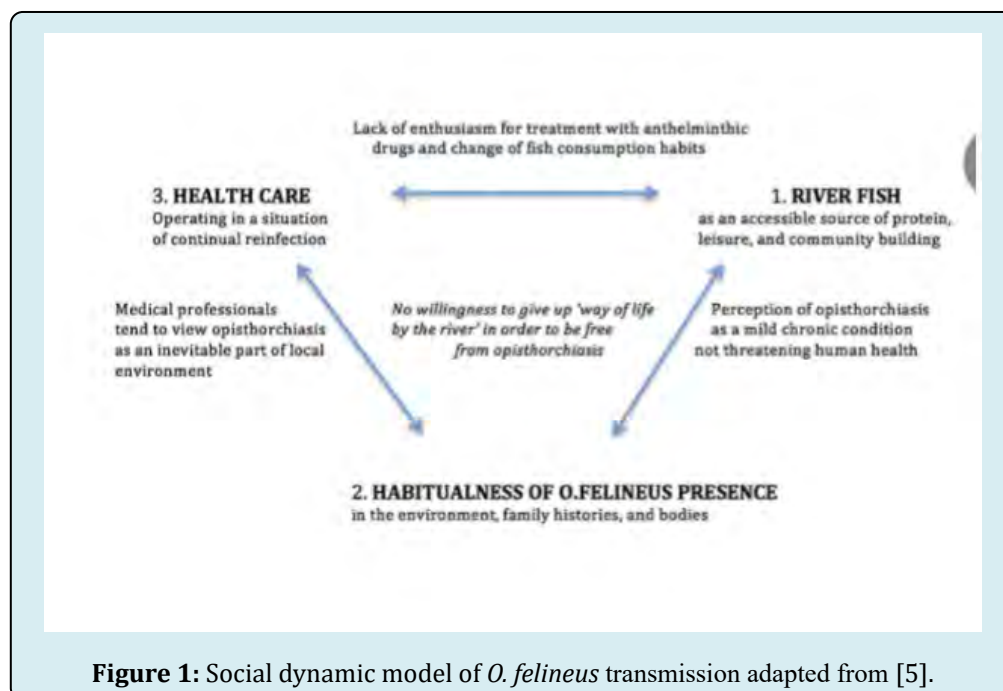
Information in different suburbs and villages of Western Siberia obtained via semi-structured interviews and questionnaires with people of indigenous background in different studies revealed that Opisthorchiasis is a well known disease by the locals and is generally regarded as a chronic mild disease by indigenous peoples [1]. This is

due to a widespread opinion in complimentary medicine that indigenous peoples have more genetic resistance to opisthorchiasis than immigrant population. An unspecified percentage of the population regard treatments (both conservative and complimentary) as more harmful than beneficial, whereas others regard them as “necessary” [2].

Knowledge of the disease does not in anyway affect the attitude of the locals to eating fish from the local river (River Ob) and very frequently (as traditional staple food) and often in raw or undercooked form hence, the prevalence of opisthorchiasis is extremely high among the indigenous people [1,6].

Indigenous knowledge on prevention and treatment options for opisthorchiasis include Thermal processing of fish (boiling, freezing, frying), salting. Thorough washing of hands, dishes and surfaces that have come in contact with fish and disinfection. Bogsphagnum was said to be good natural antiseptic for cleaning surfaces. Local medicine such as swallowing of gallbladder of pike, drinking extract of pinenuts in alcohol, or extract of aspen bark can help alleviate opisthorchiasis.

These model summaries are: (1) that opisthorchiasis is a disease of degree, i.e. opisthorchiasis becomes a disease only when there are too many parasites in the body (infection is not equal to Disease), and (2) that use of anthelmintic drugs is not necessary in Western Siberia which is a total deviation from what is observed with chronic infection of *O. viverrini* and *C. Sinensis* in Northeast Asia [6] (Figure 1).



Life Cycle

Feeding from raw or undercooked fresh water fish carrying the metacercariae of *O. felineus* results in human infection [4,7,8] (Table 1).

<i>Opisthorchis viverrini</i>	
Latin name	Local name
Cyclocheilichthys apogon	Sai Tan Ta Daeng
Labiobarbus leptocheilus	Sa
Puntius brevis	Taphian Jut
Barbonymus gonionotus	Taphian Khao
Henicorhynchus siamensis	Soi Khao
Henicorhynchus ornatipinnis	Soi Peek Daeng
Hampala dispar	Kra Sup Jut
Hampala macrolepidota	Kra Sup Khit
Osteochilus hasselti	Soi Nokkhao
<i>Opisthorchis felineus</i>	
Latin name	Common name
Leuciscus idus	Ide
L. cephalus	Chub
L. leuciscus	Dace
Rutilus rutilus	Roach
Blicca bjorkna	Silver bream
Carassius carassius	Crucian carp
C. auratus gibelio	Silver crucian carp
Abramis brama	Common bream
A. ballerus	Blue bream
A. sapa	White-eye bream
Chondrostoma nasus	Nase
Scardinius erythropthalmus	Common rudd
Pelecus cultratus	Ziege
Cyprinus carpio	Common or European carp
Tinca tinca	Tench
Gobio gobio	Gudgeon
Alburnus alburnus	Bleak
Phoxinus phoxinus	Minnow
Ph. chekanowskii	Chekanovsky minnow
Aspius aspius	Asp
Barbus borysthenticus	Barbel
Leucaspisus delineatus	Sunbleak
Cobitis taenia	Spined loach

Table 1: Local Name and Common Name of fish intermediate hosts of the Liver fluke Culled from [9].

The total list of definitive host species for this parasite

comprises 33 species and subspecies of mammals, first and foremost, belonging to the order Carnivora: domestic cats, dogs, wolves, foxes, bears and badgers. Hence, it is also known as Cat Liver Flukes [1,10]. The ingested larvae develop further and moves to the bile ducts by chemotaxis, where the biliary epithelia and biliary contents of the gall bladder is fed upon by the adult worms. The larvae are well prepared to survive the harsh weather condition as they contain, miracidia, which allows for maintaining viability in freshwater aquatic bodies for up to 3 months if they get into these bodies in the early part of spring. Moreover, they are able to survive the period of water freezing–thawing during the overall winter and retain their viability to the July of the next summer if they entered these water bodies in the late fall [1].

After about 25-30 days, the adult worms reach sexual maturity (maritae) and there is production of numerous eggs by the adult worms which enter the gastrointestinal tract and are passed out alongside faeces to the outside environment [1,11,2,4]. These eggs released find their way into the waterbodies and are ingested by freshwater snails of the family Bithyniidae. Before the mid 20th century, Bithynia leachi was regarded as the only first intermediate host for *O. felineus*; later, it was discovered that there are four species: *B.leachi*, *B.troscheli*, *B. inflata* and *B. sibirica*.The first three species are susceptible to *O. felineus*.

The *O. felineus* miracidia passes through several stages of development via asexual development (transforming through sporocysts to rediae) in the snail, which is typical of all Opisthorchiidae. Plethora of free-swimming cercariae are produced from the rediae of *O. felineus*, which leave the snail for their second inter-mediate host and, following the release of the miracidia from the eggs, for the next 3 weeks to 2 months, several development stages take place within the snail until cercariae have developed. Shed cercariae can penetrate freshwater fish, encystation takes place and are localized to the subcutaneous fat and muscle tissues at a depth of 1-2 mm. However, localization to unusual sites such as gills, gut walls or pectoral fins are not uncommon [11].

The *O. felineus* metacercariae are tolerant to drying, high salt concentration, and low temperatures and can be killed only by a high temperature [1]. According to Pakharukova and Mordvinov the typical sources of *O. felineus* infection in Russia are undercooked, sun-dried, light-salted, and light-pickled fish [1]. In addition, indigenous people of Western Siberia traditionally eat cold-served slices of frozen fish [1,2].

Within the genus *Opisthorchis*, *O. felineus* species has the highest zoonotic potential, which has important implications in terms of maintenance of transmission to humans even under high hygienic standards in which the

risk of freshwater contamination by human faeces is low as well as in veterinary medicine [12]. The morbidities associated with opisthorchiasis are largely hepatobiliary, specifically stemming from cholangiofibrosis and cholangitis, hepatobiliary inflammation, cholangiocyte dysplasia, intraepithelial neoplasia, and are expressed in a variety of manifestations, such as abdominal pain, nausea, obstructive jaundice hepatomegaly and even cholangiocarcinoma

among chronically infected individuals in a pathway similar to that caused by *O. viverrini* and *C. Sinensis* [2,4]. Cholangiocarcinoma, a generally incurable and, hence, fatal bile duct cancer [3,12], which has resulted in the classification of these parasites to the Group 1 carcinogens by the International Agency for Research on Cancer (IARC) especially in endemic regions [13-15] (Figure 2).

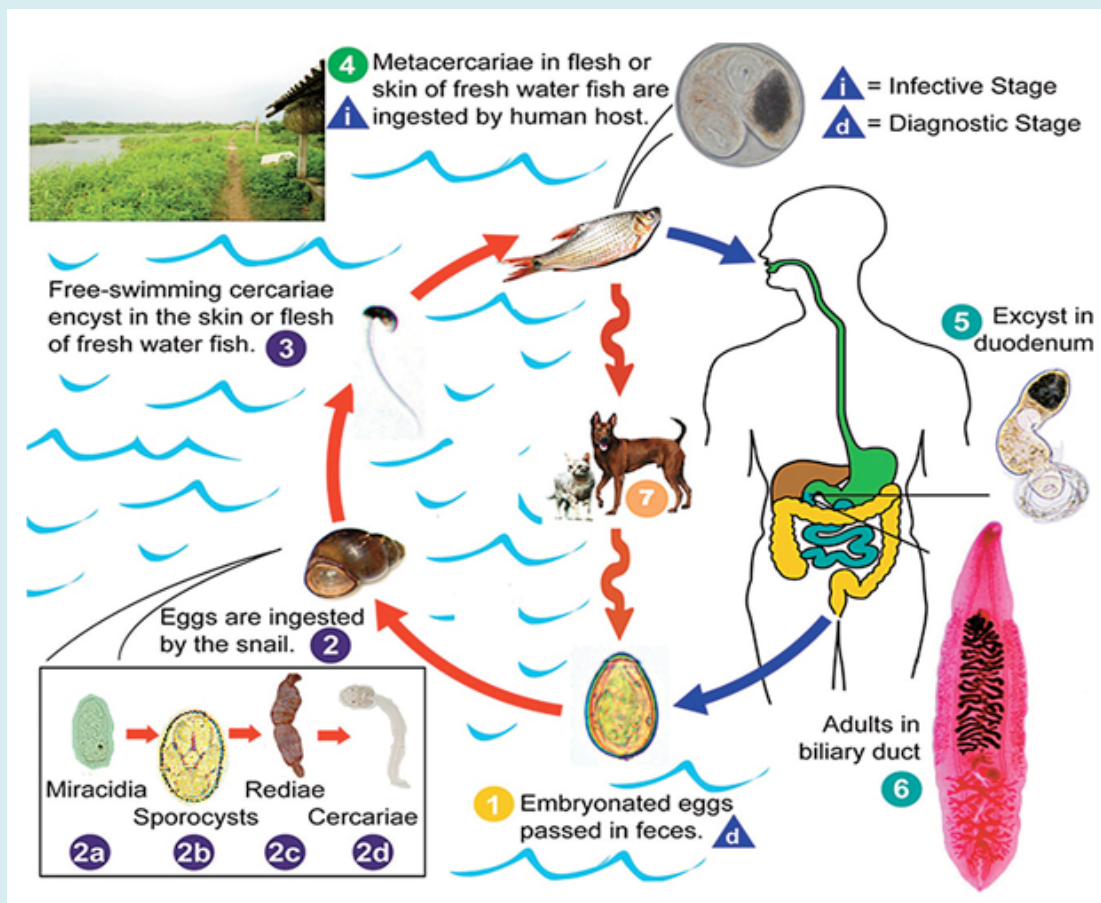


Figure 2: The life cycle of *Opisthorchis felinus* as a representative for Fish-borne Zoonotic Trematode (FZT) species [16].

Pathophysiology of Opisthorchiasis-Associated Cholangiocarcinoma

Acute inflammation of the hepatic bile ducts and portal connective tissue are the first effect of acute infection caused by Opisthorchiasis [17]. However, several studies on animal models have proven that multiple stages of carcinogenesis is an offshoot of chronic infections and inflammation. For example, In a model of experimental infection of a hamster with *O. felinus*, there was a clear cut evidence of precancerous lesions, e.g., inflammation, fibrosis, and biliary intraepithelial neoplasia of up to the III grade [17,18].

In long term infections and inflammations, histopathologically changes such as hyperplasia, adenomatous formations, and granulomatous inflammation can be seen in the bile duct epithelium via different microscopic methods [19]. The aetiology for these is due to mechanical damage and metabolic products such as oxysterol, catechol estrogen quinone-like etc excreted by the liver flukes which are injurious to the cell of the host. Mechanical injury occurs during the processes of feeding and migration, leading to ulceration of the periductal tissue. This allows for the infiltration of fluke eggs into the tissue resulting in granulomatous inflammation of the tissue

around them. Hyperplasia of the bile duct epithelium may be induced as a result of interaction with fluke metabolic products which may be immunogenic or toxic [19-31].

Infiltration of inflammatory cells has been linked to the presence of specific antigens of the liver flukes [29]. It's been hypothesized that this inflammation could be mediated by parasite-specific inflammatory cytokine interleukin (IL)-6 and later it was demonstrated that elevated IL-6 to *O. spp* excretory/secretory substance levels were linked to an increased risk of 63% for developing advanced periductal fibrosis (APF) and that individuals in the third quartile of IL-6 production had a 127% higher risk of developing advanced periductal fibrosis (APF) than individuals in the first quartile of IL-6 production [4]. It is now well established that chronic inflammation caused by long-term or recurrent *O. spp* infections plays a large part in the development of many of the factors and events that lead to cholangiocarcinoma, most notably due to the surge of production of nitric oxide (NO) [20,26-38].

Reactive nitrogen species, such as nitric oxide (NO), and reactive oxygen species such as superoxide (O_2^-) are produced in chronically inflamed tissues (using inducible NO synthase, or iNOS, in the case of NO) as an immune response aimed at

killing the parasite [20,24-26]. Immune mediated response to the parasites activate cells such as neutrophils, eosinophils and macrophages in the early stage while mononuclear subtypes including lymphocytes, macrophages, plasma cells and mast cells to synthesize NO in the later stages, which is both cytotoxic and genotoxic [24-31]. NO can react with O_2^- to produce (ONOO⁻) peroxynitrate, a more reactive molecule that can cause oxidative and nitrative DNA damage through the formation of DNA adducts such as 8-oxo-7,8-dihydro-2'-deoxyguanosine (8oxodG) and 8-nitroguanine [4].

This resultant DNA damage has been linked to an indirect cause of carcinogenesis via an increase in cell proliferation caused by proliferating cell nuclear antigen (PCNA) accumulation in the bile duct epithelium (more common with recurrent infections) [31-38]. As well as a direct causal link between NO and carcinogenesis through inhibition of DNA repair and apoptosis. Bursts of NO produced, as a result of chronic opisthorchiasis infection and inflammation, may also increase the potential for the endogenous synthesis of N-nitrosamines, such as the carcinogenic N-dimethylnitrosamine (NDMA) [24-34]. This reaction occurs within the inflamed bile duct [24]. NDMA levels is a well known biomarker as it is often detected in the urine of infected individuals.

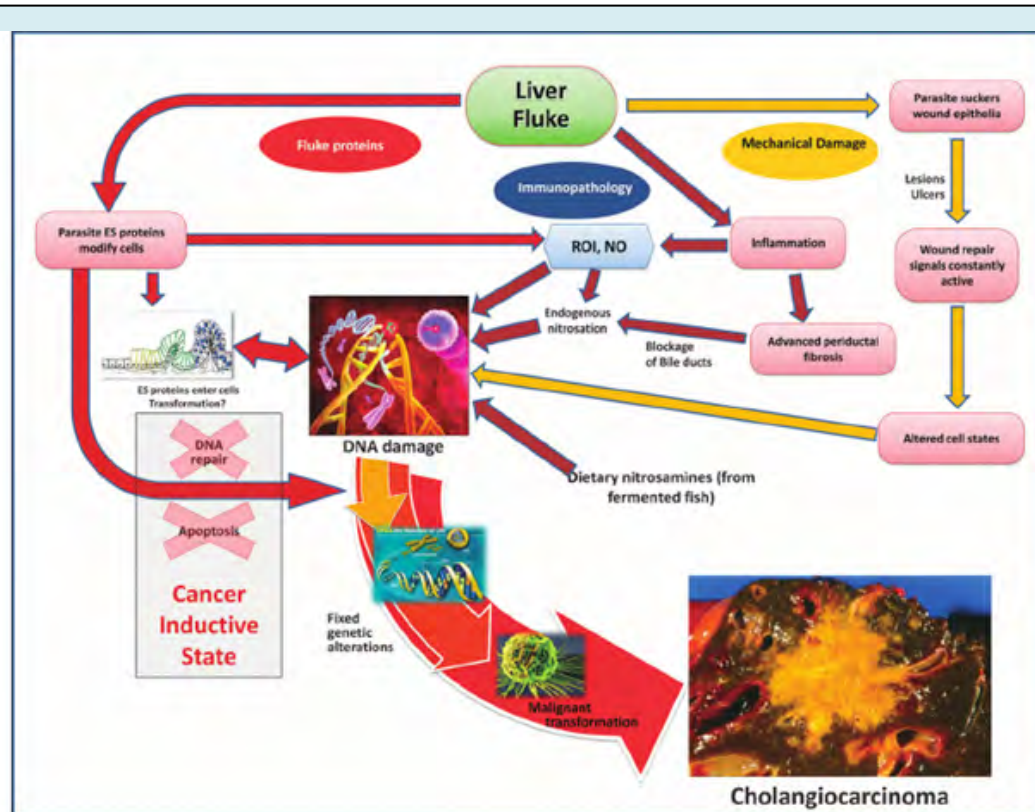


Figure 3: Hypothesized pathways of pathogenesis of opisthorchiasis-associated cholangiocarcinoma. Adapted from ref [36].

Lipid peroxidation (LPO) is another effect of inflammation-linked ROS/RNS [20,35]. The harmful effects of lipid peroxidation can be linked to cytotoxic and genotoxic properties of the secondary aldehyde species (4-Hydroxynonenal (HNE) and Malonaldehyde (MDA) that result from lipid peroxidation chain reactions. Products such as etheno-DNA adducts (ϵ dA) 1,N⁶-etheno-2'-deoxyadenosine and (ϵ dC) 3,N⁴-etheno-2'-deoxycytidine which are products of the reaction of DNA bases with the oxidized lipids (such as trans-4-hydroxy-2-nonenal) [33-38]. These oxidative lesions can initiate carcinogenesis through specific base pair substitutions and have been shown to serve as biomarkers in the leukocytes of Opisthorchiasis infected individuals, alongside 8-oxodG [38] (Figure 3).

Diagnosis

Detection of fluke eggs in stools represent the gold standard method and definitive diagnosis of opisthorchiasis. However, it's becoming increasingly unreliable in cases of low worm burden. More than one stool sample may be needed to identify the eggs. The eggs of *Opisthorchis* are very similar to those of *Clonorchis*, another liver fluke, but can be distinguished by microscopic features. This is because the method relies on the parasites having reached maturity level required to release eggs into the gastrointestinal tract, which leads to a low specificity as eggs may not be present despite an infection [4].

Monoclonal antibodies enzyme linked immunosorbent assay have been used to detect *O. spp* antigens in the feces, or coproantigens, and have been proven to be more sensitive than microscopy as it does not cross-react with flukes, when compared with indirect antibody enzyme linked immunosorbent assay, but it only detects current infections, while indirect antibody ELISA can detect past infections, as antibodies persist in body fluids following treatment with praziquantel. Indirect antibody ELISA is used when only a blood or urine sample is available. While antibody levels are higher in serum, urine is a noninvasive alternative that does not require rapid processing or a trained phlebotomist [21].

Molecular detection on faecal samples by Polymerase Chain Reactor have been described in different researches in several parts of the World. At the Tomsk Opisthorchiasis Consortium (TOPIC) held in Tomsk, Russian Federation [2]. Two molecular techniques were highlighted which includes the Taqman-based real-time PCR which was reported to have a high degree of specificity and sensitivity when working with faeces collected from experimental animal models infected with local strains of *O. felineus*. This is similar to results obtained from the molecular diagnosis of *O. viverrini* and *C. sinensis* in different studies and reliable diagnostic differentiation of *O. felineus* infection. Providing

point of care diagnostic assays is still a vision for healthcare researchers [2].

Loop-mediated isothermal amplification (LAMP) method is the second molecular diagnostic techniques that have been proven to be faster when compared to the traditionally used PCR, it is sensitive, specific and requiring minimal processing and instrumentation, with results available by reading with the naked eye. This method has been used for the detection of the *O. viverrini* and *C. Sinensis* and its application to detection of *O. felineus* is being established [2].

Additionally, use of imaging techniques such as Ultrasound, Computer Tomography (CT) and Magnetic Resonance Imaging (MRI) can sometimes reveal cysts containing the parasite. Imaging methods are also used to determine the extent of the hepatobiliary disease [20]. Both CT and MRI can determine malignancy, but MRI provides superior soft-tissue resolution, can determine periductal infiltration and the extent of localization of a tumor, and can identify small lesions to a higher degree than CT. An endoscopic retrograde cholangiopancreatography (ERCP) is used to image the biliary tree while also obtaining biliary brushings and bile samples for histopathology and cytology. Magnetic resonance cholangiopancreatography (MRCP) is a magnetic resonance imaging scan optimized for the biliary and pancreatic ducts and is increasingly preferred as the diagnostic test of choice due to its noninvasive nature as opposed to ERCP. All current available biomarkers lack specificity and sensitivity [4].

Treatment

The main treatment for Opisthorchiidae is based on giving praziquantel at 25-40 mg/kg body weight [17] some researchers have suggested the use of albendazole at 10 mg/kg body weight. Praziquantel is marketed under many brand names depending on the manufacturer, for example Biltricid and Droncid from Bayer for clinical and veterinary uses, respectively. Data from studies on praziquantel in the *O. felineus* animal model and in vitro on juvenile and adult worms, have revealed the effectiveness of the praziquantel drug. The mode of action of this drug is that it disrupts calcium ion homeostasis and antagonizes voltage-gated calcium channels leading to distortion of parasite motility, viability, tegument damage as well as death of the parasite [1,2,4,5].

However, research have shown that without praziquantel treatment, 8-nitroguanine and 8-oxodG were shown to remain present in the epithelium of hamsters six months after they were infected [20]. The amount of the DNA adducts is also significantly lowered by antiparasitic drug treatment and can also be measured in the urine [4].

However, reinfection was shown to increase the rate of inflammatory cell infiltration, iNOS expression in the epithelium, production of NO, and formation of 8-nitroguanine and 8-oxodG with each reinfection-ultimately increasing DNA damage and risk of cancer development [4]. This raises the question as to whether or not it is best to treat individuals who are at a high risk of reinfection with praziquantel, as it could do more harm than good. There are many societal, ethical and legal complications to this question. However, until this question is answered, it is suggested that praziquantel must be given to only those who are not at risk of reinfection [4].

Prevention

The campaign for reduction of raw fish consumption among the aboriginal population of Western Siberia to date has proven ineffective and difficult to implement in practice as it always met with stiff resistance as inherent culture is really difficult to change. This is further complicated by the high sensitivity and specificity detecting infected cases that invariably have light infection (egg g^{-1} feces < 1000) as well as the problem of reinfection highlighted earlier. Despite the huge amount of people infected and at risk of infection, in addition to the economic losses resulting from liver fluke infection, require the devotion of time and resources to develop and implement control and prevention measures. Ideally, improvements in hygiene and sanitation practices prevent transmission of eggs from feces, which disrupts the cycle of fluke maturation and transmission. To be successful and long-lasting, prevention programs require broad community acceptance and participation.

The easiest and most promising way of preventing opisthorchiasis is decontaminating fish, as metacercariae are the only source of infection for humans. A multitude of studies have examined the viability of metacercariae under various environmental conditions and it is known that they remain viable when frozen at -20°C for up to 30 days. Temperatures of $>50^{\circ}\text{C}$ kill metacercariae in a few minutes, however, it is generally recommended to cook fish in boiled water for more than 30 mins to ensure that metacercariae are killed in frozen pieces of fish they must be cooked for a long time. Very popular fish dishes of dried and salted fish are not safe for consumption. Salted fish is generally considered ready in 24-48 hrs. It has been proven that metacercariae remain viable under high salt concentrations for up to 14 days. Therefore, in this regard, the popular dish of the Western Siberia in Russia and eastern Europe of slightly salted fish is not safe at all. The same is true for dried fish where greater than 12 days of drying are required to kill 99% of meta-cercariae. Given that the weight of fish and the temperature of drying are not constant even at fish plants or after 12-day period, dried fish remain dangerous for consumption. Hot smoking is a reliable method of fish decontamination. A period of 2-2.5 hrs at 70-

80°C kills all metacercariae. Alternatively, cold smoking has similar effects to those of drying and salting. The same is true for treating fish with vinegar where it has been shown that 100% of metacercariae remain viable and infectious after 24 hrs.

Control

Interrupting the life cycle of the parasite has always been regarded as a promising way of disease control, for example application of molluscicides to control snail populations. Low concentrations of certain molluscicides (e.g., phenasal and niclosamide) are lethal for infected snails, sublethal for uninfected ones, and presumably nontoxic for other animals. Before the discovery of praziquantel anti-helminthic activity, application of molluscicides was practiced for some time in Russia. The disadvantage of this approach is its adverse effect on the ecology of the water body. The economic feasibility of using molluscicides is only practical for small water bodies since the costs of treating big areas are extremely high and do require repeated treatments as it has been shown that snail populations are restored in about 5 years. Therefore, decontamination of aquatic bodies has been abandoned in Russia [9].

The control efforts are primarily focused on the reduction and elimination of parasite transmission by ensuring proper food preparation, promoting the development of improved diagnostic techniques, providing chemotherapy, and improving sanitation. A combination of health education, mass treatment, and governmental aid could significantly reduce liver fluke infection. Emphasis on health education is also been placed on the younger generation and foreigners in school as a part of the conventional curriculum. So far, this has been very effective as there are increasing number of foreign students in the Western Siberian region particularly in Tomsk region which play hosts to seven Universities, most of which have different program for International students and has no known record of any of International students suffering from Opisthorchiasis.

The possibility of developing a vaccine against liver fluke is not such a easy task but this approach is been pursued by researchers although no success has been recorded thus far. The genome mapping for all the Liver Flukes is expected to facilitate vaccine development and tests for its control. To ensure the success of liver fluke control programs, trans disciplinary approach is required.

Recent Public Health Interventions For *O. Felineus* in Western Siberian Region

As at present, no continuous disease control programme has been developed and implemented in the region by both

the regional government of the affected regions (Western Siberian) as well as the central government of the Russian Federation this may not be unconnected with the fact that Opisthorchiasis caused by *O. felinus* is a chronic illness and even asymptomatic in a sizeable number of population [4]. However a lot of researchers of local and international repute are beginning to pay attention to this neglected disease. Hence different models are been proposed including the Social Dynamic Model (SDM) discussed earlier in this article. The programme puts into consideration sociocultural processes that animate the transmission of the *O. felinus* parasite and the specificities of the local context where intervention is planned and not only the biomedical components of the disease. This is similar to such integrated approaches illustrated by; for example the history of opisthorchiasis control in Thailand [39] and by the experiences with interventions for zoonotic tapeworm *Taenia solium* in Northern Lao People's Democratic Republic [40].

Noteworthy is the attention paid by scientists in the mid part of the last decade from Russia, Southeast Asia, Europe, and the United States who came together in a city in Western Siberia (Russia) to form a Tomsk Opisthorchiasis Consortium (TOPIC). The initiative was designed to strengthen integrated control, to conduct research and to raise awareness on Opisthorchiasis afflicting populations not only in the tropical regions of East Asia but also in temperate and semi-arctic areas of Europe and Asia [1]. The TOPIC consortium adopted the "Lawa model," as a template which has been successfully used previously and is based on using the EcoHealth approach at the lake Lawa region of Northeast Thailand [39]. This method is to be modified according to local health care systems and cultural sensitivities in other endemic areas, but not differing in essence of involving trans-disciplinary and stakeholder participation at every level. This initiative was supported as Private and Public Partnerships. However, there is still much to be done in translating research and theoretical approaches into practical realities in the Public health Intervention for this chronic disease.

Conclusion

Despite the significant global burden of Opisthorchiasis, it is still one of the most neglected disease probably due to the fact that it is responsible for a chronic illness, however it's time to start putting on the spotlight on it, one of which the publication aims to achieve with much emphasis on its distribution in the Western Siberian region.

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