Acta Tropica xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

Towards improved diagnosis of neglected zoonotic trematodes using a One Health approach☆

Maria Vang Johansen[a,*], Tore Lier[b], Paiboon Sithithaworn[c]

[a] Section for Parasitology and Aquatic Diseases, Department of Veterinary Disease Biology, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark
[b] Department of Microbiology and Infection Control, University Hospital of North Norway, Norway
[c] Department of Parasitology and Liver Fluke and Cholangiocarcinoma Research Center, Faculty of Medicine, Khon Kaen University, Thailand

ARTICLE INFO

Article history:
Received 19 February 2013
Received in revised form 2 July 2013
Accepted 13 July 2013
Available online xxx

Keywords:
Diagnosis
Trematode zoonoses
Opisthorchis viverrini
Clonorchis sininsis
Schistosoma japonicum
Schistosoma mekongi
Neglected Tropical Diseases
One Health

ABSTRACT

Reaching the goal of control, elimination and eradication of the Neglected Tropical Disease in a foreseeable future provides significant challenges at the ground level especially regarding helminthoses. Helminths are still mainly diagnoses by egg identification in stool, methods with low sensitivity and for most species low specificity. Cross-sectoral collaboration with regard to zoonoses is almost non-existing and cross validation by inter-laboratory evaluation of diagnostic tests is not a common practice. The aim of this review was to elucidate the dilemma of helminth diagnosis using zoonotic trematodes as examples. Much progress has been made improving the diagnostic sensitivity of *Opisthorchis* and *Clonorchis* using DNA-based techniques but the specificity of these tests is still a challenge due to the many most common but neglected intestinal trematodes. The burden of these diseases and ways to control them remains to be elucidated. Although efficacious drugs are available, the effectiveness of mass drug administration remains to be assessed. The importance of animal reservoirs and ways to control the diseases in animals are yet unknown. Diagnostic challenges regarding *Schistosoma japonicum* and *Schistosoma mekongi* include the many light infections and the persisting influx from the animal reservoirs. The sensitivity of the faecal based techniques suited morbidity control but will be insufficient for elimination of the helminths. More accurate diagnostic tools are required and new algorithms for detection and progression of helminth elimination will be needed. Standardized inter-laboratory test validation, inter-sectoral collaboration and establishment of an international One Health diagnostic platform, sharing best practices on diagnosis of helminth zoonoses, could all significantly contribute to control and elimination of these diseases.

1. Introduction – from microscopes to molecular bands

In the second WHO report on Neglected Tropical Diseases (NTD), a detailed roadmap is outlined for control, elimination and possible eradication of the 17 identified NTD before 2020. The roadmap is a very promising effort initiating concerted action against the NTD which currently affects nearly 2 billion people Worldwide (WHO, 2013). The strategy combines five intervention strategies (i.e. preventive chemotherapy, intensive case management, vector control, improving water and sanitation and including veterinary public health) of which preventive chemotherapy is being promoted as the most important for many of the NTD (WHO, 2013). However the report also urges the need to refine strategies, and develop new tools and algorithms as the outlined plan causes major challenges for most existing systems. A central prerequisite for control of the NTD is accurate diagnosis and in the case of the neglected zoonoses, techniques for animal diagnosis also need to be available but as pointed out in the report by WHO (2010) major challenges exist regarding proper diagnostic and assessment tools for many of the NTD. Zoonotic trematodes like foodborne zoonotic trematodes and Asian schistosomiasis serve as good examples. Major knowledge gaps exist regarding the burden of these zoonoses and for the foodborne zoonotic trematodes even species identification is a problem. Without these, identification of the problem as well as assessment of any intervention effect will remain a challenge (Fig. 1).

Zoonotic trematodes, like most helminths, are diagnosed today in man and animals primarily by microscopy of helminth eggs in stool; a method introduced in the late 19th century. Immunological

* Corresponding author at: Department of Veterinary Disease Biology, Faculty of Health and Medical Sciences, University of Copenhagen, Thorvaldsensvej 57, DK-1871 Frederiksberg C, Denmark. Tel.: +45 35 33 14 38.
E-mail addresses: mvj@sund.ku.dk, mvj@bilharziasis.dk, mvj@life.ku.dk (M.V. Johansen).

0001-706X/$ – see front matter © 2013 The Authors. Published by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.actatropica.2013.07.006

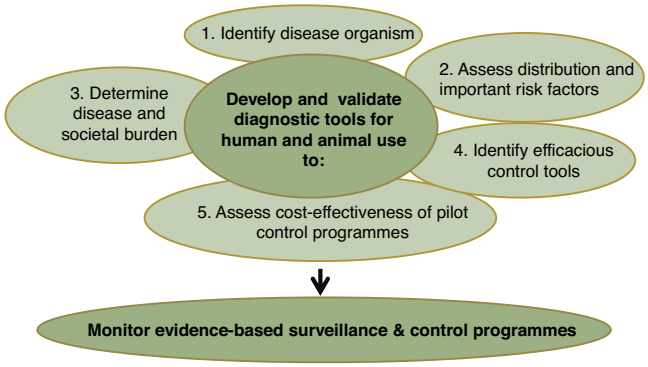

Fig. 1. Illustration of essential steps from parasite identification to evidence-based surveillance and control. Each step will require development and validation of appropriate diagnostic tools for both human and animal hosts.

tests, numerous and diverse, are generally less used and less validated. Only in very recent years have molecular techniques been introduced and are now being optimized and validated across country borders. As the clinical manifestations for zoonotic trematodes are non-pathognomonic, clinical diagnosis makes little sense. The lack of a reliable gold standard has been another major drawback in diagnostic advancements of these parasites. As the new tests are being evaluated against a suboptimal gold standard, the outcome of the assessment remains questionable (Fig. 2). This has been an on-going debate while trying to introduce serological tests assessing specific antibodies as well as molecular methods. While some attention has been paid to develop and improve diagnostic tools for human zoonotic trematodes, the diagnostic ante mortem opportunities for animal zoonotic trematodes is almost non existing despite a growing recognition of their importance in control and eventually eradication of these diseases. In the following an update on the diagnostic advancements and present challenges are presented for three groups of neglected zoonoses among the NTD.

1.1. Opisthorchiasis and clonorchiasis

The foodborne zoonotic trematodes consist of liver, lung and intestinal flukes of which the most important species parasitizing humans are the fishborne zoonotic trematodes *Opisthorchis viverrini*, *Opisthorchis felineus* and *Clonorchis sinensis* (Keiser and Utzinger, 2009; Sithithaworn et al., 2012). Distribution of these liver flukes is focal in nature and restricted to areas where the first and second intermediate hosts are abundant. In addition, their distribution ranges are related to habit of eating raw, pickle, or undercooked fish and fishery products particularly in Southeast Asia (Grundy-Warr et al., 2012). Distribution ranges of these liver flukes do generally not overlap for example *O. viverrini* is known to occur in Thailand, Lao PDR, Cambodia and Vietnam while *C. sinensis* is endemic in China, Korea, Taiwan, Vietnam and part of Russia (Sithithaworn et al., 2012). *O. felineus* confine to Eurasia in the former Soviet Union, Kazakhstan, the Ukraine and Europe such as Italy and Germany (Pozio et al., 2013). Recent outbreak of *O. felineus* in Bolsena and Bracciano lakes in central Italy demonstrated an ongoing maintenance of life cycle of this trematode in animal reservoir hosts in Europe (De Liberato et al., 2011).

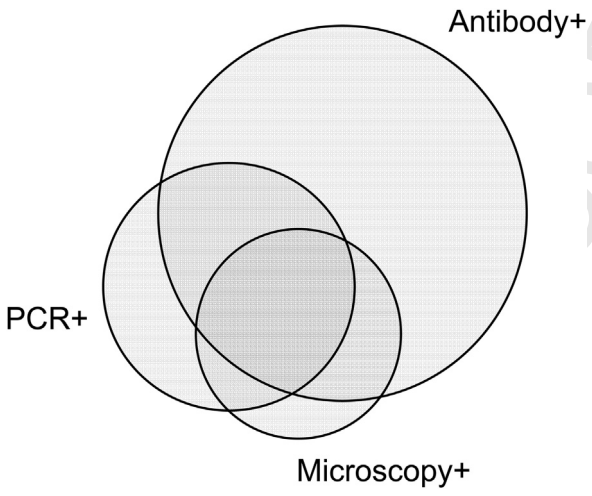

Fig. 2. Example of combinations of test results. The accuracy of a new test (here PCR) will be wrongly perceived as low if a test with low sensitivity (here microscopy) or low specificity (here antibody detection) are used as gold standard.

The currently available diagnostic methods for liver fluke detection are still far from ideal and significant problems are seen in areas with low prevalence, light infections and co-infection with other trematodes. In addition, diagnosis problem persists due to widespread use of chemotherapy for parasite control and this may increase proportion of light infection individuals. In the following different approaches will be presented and discussed.

Conventional faecal examination as well as recovery of adult worms is still the gold standard diagnoses of *O. viverrini*, *O. felineus* and *C. sinensis*. Eggs can be detected either in faeces, bile from nasobiliary or percutaneous transhepaticbiliary drainage (PTBD) during treatment of bile duct obstruction, or duodenal fluid. Adult worms may be encountered from expulsion chemotherapy (Ramsay et al., 1989; Elkins et al., 1991; Radomyos et al., 1994; Joo and Bang, 2005) or more accurately from the liver at post mortem examination (Sithithaworn et al., 1991). Faecal examination is routinely used for diagnosis of liver fluke infections due to non-invasiveness and ease of sample collection. Commonly used techniques include the modified formalin ether (or ethyl acetate) concentration technique (FECT) (Elkins et al., 1990), the modified Kato-Katz thick smear (Hong et al., 2003), and Stoll's dilution egg count technique (Viyanant et al., 1983). These classical coprological techniques all have major problems regarding specificity and sensitivity.

Under light microscopy, the eggs of the liver flukes are characterized by rough and thick egg shells, and are similar in shape and size to several species of food-borne trematodes belonging to the families Opisthorchiidae, Heterophyidae and Lecithodendriidae (Figs. 3 and 4). The latter two families of trematodes are collectively referred to as minute intestinal flukes (MIF) because of the small size of the adult worms compared to the liver flukes (Kaewkes, 2003; Chai et al., 2005; De and Le, 2011). Eggs of Opisthorchiidae and Heterophyidae are very similar while eggs of Lecithodendriidae do have smooth egg shall and less distinct shoulder in comparison with other two groups (Kaewkes, 2003). The marked morphological resemblance of MIF eggs with those of *O. viverrini* and *C. sinensis* increases the probability of a false positive diagnosis, with concomitant decrease in diagnostic specificity.

As the parasitological techniques rely on egg excretion, they cannot detect infection during prepatency, and they have poor sensitivity in light infections, in old chronic infections, following treatment, or when the bile duct is obstructed causing intermittent egg excretion. Repeated examinations are needed to improve diagnostic sensitivity of faecal examination. Three consecutive Kato-Katz thick smears were reported to be more sensitive than a single examination by FECT (Lovis et al., 2009). However, even with repeated stool examination using a standardized method like FECT, discrepancy remains between egg count and worm detection. For example in an autopsy study, adult *O. viverrini* were recovered from 113 of 139 (81.2%) livers but faecal examinations of the same individuals determined that only 86 cases (67%) were egg positive by FECT (Sithithaworn et al., 1991). From this study, the detection limit by conventional faecal examination methods was estimated to be