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EDITORIAL

Immunodiagnosis of Human Liver Fluke Infections

Liver fluke infection is still an important endemic disease in many parts of the world. It is not unrealistic to estimate that at least 40-50 million people are currently being infected, and in some areas, the incidence is increasing. The three important parasites causing the disease are *Clonorchis sinensis, Opisthorchis felineus* and *Opisthorchis viverrini.*[1,2] Clonorchiasis is prevalent in China, Hong Kong, Korea, Taiwan, North Vietnam and Japan. It was estimated that more than 19 million people are infected with *C. sinensis.* Opisthorchiasis caused by *O. felineus* has been reported from the U.S.S.R. and central and eastern Europe. There are probably several million cases of *O. felineus* infection with a prevalence rate as high as 85% in some areas. On the other hand, *O. viverrini* infection in humans is found mainly in the northeastern and northern parts of Thailand, and in Laos, Cambodia and parts of Vietnam. Opisthorchiasis caused by *O. viverrini* is one of the diseases of public health importance in Thailand, as it has been estimated that at the present time there are at least 7 million people infected by this parasite, representing approximately 15% of the total population. The prevalence of *O. viverrini* infection among the population in northestern Thailand has risen from 3.5 million cases in 1965 to 5.4 million cases in 1981.[3] In some villages in the endemic areas, a prevalence of more than 90% has been reported. All age groups including infants are known to be infected.[4]

In the past, both opisthorchiasis and clonorchiasis have been reported sporadically from non-endemic areas including North America and Western Europe but at the present time increasing number of cases have been diagnosed. One main reason is due to ever increasing number of immigrants and refugees from less-developed countries within the last few years.[5-8] Another possible reason is the importation of fish from the endemic areas. For example, the disease has been reported in native Hawaiians as a result of consumption of infected fish imported from endemic areas.[2]

Clinical manifestations of patients infected with liver flukes vary considerbly depending on the intensity and duration of infection.[9] The majority of cases are well tolerated and asymptomatic, and are diagnosed based on routine stool examination. In those with symptoms, there may be only intermittent dull pain with slight discomfort. However, in more severe and chronic infection, there can be biliary obstruction, cholangiocarcinoma and even death.

Adult flukes inhabit the biliary system of man. The early pathological changes consist of an acute inflammatory reaction of the bile duct and the portal connective tissues.[9] Periductal inflammation with mononuclear cell and lymphocyte infiltration has been observed. These pathological changes can be the result of worm movement, of toxic metabolites released from the flukes or of immunological response of the host. The latter has been amply demonstrated, particularly with regard to the humoral immune response. On the other hand, immunopathological changes similar to egg granulomas in schistosomiasis have never been well documented in the liver fluke infection.

Current methods for the diagnosis of human liver fluke infection are based on the demonstration of eggs in either stool, duodenal fluid or bile.[1,4,10] Although such an examination is reliable and permits the identification of species, it is unfortunate

that such a technique is useful only when the intensity of infection is high and is reliable only in the hands of experienced personnel. It is easy to diagnose cases with heavy infection when several thousand eggs per g of feces are present. However, the diagnosis of light infection based on stool examination is uncertain, can be easily missed and may vary considerably from one sample to another. Moreover, a decision is even more difficult to make when samples are from populations with mixed parasitic infections, *i.e.*, eggs from *O. viverrini* are difficult to distinguish from those, of small intestinal flukes, particularly by those who are rather inexperienced. These flukes include, for example, *Prosthodendrium molenkampi, Phaneropsolus bonnei, Haplorchis pumilio* and *H. taichui.*[11] Moreover, in infections sufficiently severe so as to result in biliary obstruction, eggs will not be recovered in the feces. In order to identify eggs in the latter situation, one has to examine for the presence of eggs in cannulated bile. However, such a technique is not practical and is not without danger. These drawbacks can be minimized if one is aware of the problem and when experienced clinicians and parasitologists are available. However, nowadays with mass migration of people from endemic areas (*e.g.*, immigrants and refugees from Far Eastern or Southeast Asian countries) to non-endemic areas such as North America and Europe, difficulty in establishing diagnosis based on morphological stool examination for fluke eggs can be a problem. Therefore, if techniques or reagents for identification of eggs, (as by immunofluorescent antibody specific for fluke eggs or ELISA for soluble parasite antigens) or immunodiagnosis based on detection of specific antibody in serum or secretions of suspected patients are available, the problem of diagnosis should be alleviated.

Immunodiagnosis of helminthic infections

To circumvent the tedious, time-consuming and frequently impractical microscopic examination, many parasitologists have now turned to immunodiagnostic procedures such as intradermal and serological tests as well as antigen detection for many helminthic infections. Intradermal tests of both immediate and delayed types have been used in several helminthic infections. Generally, these intradermal tests have rather poor specificity and thus have limited value for the diagnosis of individual cases although they may be of some value in epidemiological surveys. Moreover, standardization and interpretation of results are not yet satisfactory.

Unlike the intradermal tests, serological tests based on analysis of serum specimens are more popular, and have been widely used in parasitological research during the last 10-20 years. Within certain limitations, serological tests are invaluable aids to diagnosis of individual cases, for epidemiological surveys, and to aid in the evaluation of control measures. Up to now most of the standard serological procedures have been used in the diagnosis of helminthic infections.[12] These include complement fixation, flocculation, indirect hemagglutination, indirect immunofluorescence, immunodiffusion, immunoelectrophoresis, countercurrent electrophoresis, radio-immunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA). In addition to these standard tests, some special tests have also been developed, *e.g.*, circumoval precipitation, circumsporozoite precipitation, " cercarienhullen " reaction, fluoroimmunoassay (FIAX) and miracidial immobilization. Newer procedures like the indium slide immunoassay have been modified for use in parasitic infections. Many of the tests mentioned above are now available commercially as preformed kits ready to be used. However, it is unlikely that others will be available in the near future as commercial enterprises are generally not too eager to develop them due to the low commercial benefit, as these diseases largely involve people of low economical status. Up until a few years ago, the complement fixation test was one of the most reliable and most widely used, for example, in trichinosis, filariasis and schistosomiasis. However, the test has to be performed under optimal conditions and requires experienced supervision. A further complication is that many antigens used, as well as a proportion of clinical specimens have anticomplementary activity. A large number of investigators have recently turned to ELISA as a possible new diagnostic tool for parasitic diseases, many of which have already been thoroughly evaluated and seem to give highly satisfactory results. Moreover, the two recent developments, namely hybridoma and recombinant DNA technique, will undoubtedly contribute to the future progress in this area of investigation.

The major problems about immunodiagnosis in parasitic diseases are their relatively poor sensitivity, low specificity and the non-availability of appropriate antigens. Nevertheless, it is to be expected that with new technology, these problems will soon be alleviated. Much attention has been given to the purification and production of sufficient quantities of antigens for the test, as both the nature and the source of these antigens contribute significantly to the success of these immunological proceduces. In the past, investiga-

tors had used the stage in the life cycle which can be most easily obtained in large quantities. However, this practice does not always provide the best antigen for immunodiagnosis. One of the main difficulties in developing reliable diagnostic tests in parasitic diseases lies in the complex nature of the antigens used for such a purpose. Complexity in this case involves not only the multicellular nature of the parasites themselves but also involves changes in the nature of antigens as the parasite develops from one stage to another in the same mammalian host. Therefore, when such a complex antigen is used for detection of antibody, phylogenetically related species, or sometimes even unrelated parasites, may give rise to cross-reacting antibodies. Attempts have been made with some success to prepare and purify antigens from phylogenetically related parasites of animals which may be obtained in much larger quantity. One should keep in mind however that the use of cross-reacting antigens may be inadequate for making reliable diagnosis.

Different parasite components have been use as antigens for immunodiagnosis of parasitic infections of man and animals. In general, these antigens are grouped into 3 main categories, namely somatic, excretory and secretory (ES or metabolic) including egg, and surface antigens.

Somatic antigens.

These antigens are highly complex. They are commonly prepared and used in the form of aqueous extracts from appropriate developmental stages that are available in sufficient quantity, *e.g.*, adult stages. After homogenization and brief sonication, the supernatant containing crude somatic conponents and stored metabolic products is dialyzed and lyophilized for storage. Some investigators have treated the worms with ether, prior to aqueous extraction, to eliminate lipids which may interfere with some serological procedures. However, these protocols sometime destroy labile parasite components. Various methods have been used to partially purify the crude somatic preparation and these include ion exchange chromatography, gel-filtration, electrophoresis, electrofocusing and alcohol precipitation. On a few occasions, affinity chromatography using enzyme inhibitors, lectins, and antibodies have also been employed to purify some special parasite components from the crude or partially purified preparations. Elimination of common or cross-reacting parasite antigens or host components has been achieved by affinity chromatography using polyclonal cross-reacting antibodies or anti-host components respectively. Recently however, opportunity to purify antigens using appropriate monoclonal antibodies is also available. The latter approach has very much simplified the purification of appropriate antigens which may be present only in trace quantity in the crude material. The availability of purified antigens has made immunodiagnosis of parasitic diseases as specific and sensitive as has been achieved with bacterial and viral diseases. Moreover, the identification of suitable parasite-specific antigens and the availability of monospecific antibodies, either monoclonal or polyclonal, open the possibility of producing diagnostic peptides containing important parasite epitopes in sufficient quantity, *e.g.*, by recombinant DNA technology.

Metabolic or excretory-secretory (ES) antigens.

As helminths develop from the infective larval stage to the adult stage, they have undergone metabolic changes during the course of infection, thus presenting different antigens to the host. It is reasonable to assume therefore, that the antibodies produced in response to these products are more specific and more closely related to active infection than those elicited by somatic antigens. The ES antigens have been used for the detection of antibodies by several standard serological procedures and for many of these infections they prove to be highly specific and are sensitive enough for immunodiagnostic purposes.[13] Chemically, they are in general less complex than somatic antigens but for those that have been well analyzed they are still quite antigenically complicated.[14] These components are largely enzymic in nature and are often the products of reproductive and digestive systems. They can be obtained in large quantity during the *in vitro* culture or maintenance of parasites in the laboratory. Some parasites produce and release sufficient amounts of ES into the culture fluid that they can be used without further concentration. One difficulty is that not all parasites can be cultured or maintained *in vitro* long enough to release sufficient amounts of ES antigens. In addition, for some parasites, protein supplement is required and may interfere with later steps of purification. These ES antgens can also be recovered in large quantity in the somatic extracts of these parasites.

In addition to these soluble metabolites, many parasites release eggs which are potent immunogens, *e.g.* those of Schistosomes. Both humoral and cell-mediated immune responses to egg antigens have been reported for several parasitic infections. These responses are also known to be associated with the immunopathology of seveal diseases, *e.g.*, egg granuloma in schistosomiasis. Moreover, the

antibodies to these antigens may be protective and are at times useful in immonodiagnosis. Antibodies to liver fluke eggs have not been successfully detected in experimentally infected rats, guinea pigs and rabbits nor in the serum of patients with clonorchiasis.[15-17] However, Flavell[18] and our group (unpublished) have shown that antibodies to egg antigens are present in the serum of infected hamsters. Because it was possible to raise antibodies to egg antigens by immunizing the animals with the egg extract, such antibodies may be of value from a diagnostic point of view, particularly if monoclonal antibodies to these antigens are available. Recently, sera from patients with opisthorchiasis were shown to react with *O. viverrini* eggs by an immunofluorescent technique.[19]

Surface antigens.

Teguments of many parasites are metabolically active. Surface protein serves as a dominant antigen and has a likelihood of stimulating the host immune system. Therefore, many investigators have used the external surface of parasites or external membranes of infected host cells for their studies. For some parasitic infections, these external surface components not only serve as host " protective " antigens but also are of value in immunodiagnosis. Various methods have been used to extract antigens from the surface of parasites or parasite-infected cells. Peripheral proteins can be solubilized with high ionic strength buffer or metal chelators. Integral membrane proteins, on the other hand, require more vigorous procedures. In general, this has been achieved with detergents, organic solvents or denaturing agents like urea or guanidine or a combination thereof. The use of non-ionic detergents is preferred by most investigators as they are mild enough not to denature these surface proteins. These extracts can be used as such or can be fractionated further via, for example, affinity chromatography, high performance liquid chromatography or isoelectrofocusing.

Although the above classification of parasite antigens may seem to be straightforward, a wide grey area exists. Common antigens have been repeatedly demonstrated among phylogenetically distinct species of parasites, among different developmental stages of the same or related parasites, and among different tissues in any one developmental stage.[20-24] The wide specificity that exists suggests the existance of common antigenic pools having small antigenic determinants with simple molecular configuration, as has been noted in malaria.

Assuming that reliable antigen is now available, the next step is to select an appropriate serological test. Unlike bacterial and viral infections, parasitic infections are more insidious and chronic in nature. Therefore, detection of IgM antibody is rarely needed and most tests involve the detection of IgG antibody. If the antigen is not pure enough, then cross-reaction with other parasitic infections can be a problem. The latter could however be minimized if other antibody isotypes are determined instead of IgG. For instance, for helminthic infections which generally have a potentiated IgE response, detection of specific IgE antibody may be more useful. For mucous surface membrane infections, the detection of IgA or IgE antibodies may be more specific than that of IgG antibody and is therefore more useful diagnostically. On occasion, however, the high concentration of IgG antibody present in the serum of these patients may interfere with the quantitation of IgA or IgE antibody. In such a situation, certain modifications have to be made to minimize this. When appropriate, one can use other fluids which have low concentration of IgG but higher concentration of other immunoglobulin classes. One possibility is, for these mucosal intections, to use external fluids instead of serum. Saliva can be a good candidate because it has a minimal amount of IgG and has a fairly high concentration of IgA.

Although detection of antibody is quite popular and is highly practical both for individual cases and for epidemiology surveys particularly when a paper-disc method for blood collection is used, it does not indicate active infection. All tests still give positive results soon after infection has been terminated, *i.e.*, following anthelmintic therapy. However, if a sufficient period of time is allowed before retesting, some tests will then give a negative result. For this purpose however, it is better to look for the presence of parasite antigen or immune complexes. These antigens can be detected in the circulation and urine, for example, in schistosomiasis. More frequently, they can be more easily detected in fluids within circumscribed spaces like cystic fluid or cerebrospinal fluid (*e.g.*, cysticercosis) In addition to these examples, parasite antigens have also been demonstrated in external secretions, *e.g.*, bile and intestinal secretions.[16] Many standard serological tests have been employed and these include immunodiffusion and ELISA. The concentration of antigen in these fluids depends primarily on worm burden. However, with the current serological methods like RIA or ELISA, antigens in nanogram or picogram amounts can still be detected, particularly when monoclonal antibodies to many of these antigens are or will be available in the near future.

Previous efforts to develop immunodiagnostic methods for liver fluke infections

It has been more than 20 years since investigators first attempted to develop immunodiagnostic methods for human liver fluke infections caused by *C. sinensis, O. felineus* and *O. viverrini.*[25,26] These early attempts made use of immunological procedures available at that time, *e.g.* intradermal skin tests, complement fixation, precipitation reaction, as well as various modifications of agglutination reactions. The species of parasite most intensively investigated during that early period was *C. sinensis.* Subsequently, different groups of Russian investigators have used these techniques to approach the problem caused by *O. felineus.*[27-30] On the other hand, investigation on *O. viverrini* has not started until quite recently.

The principal obstacles to progress in this area of investigation are due to insufficient knowledge concerning the immunology of these parasites, inadequate quantities of appropriate parasite antigens available for intensive investigation and strong immunological cross reaction between these flukes and other parasites, some of which are phylogenetically unrelated to them. The last problem can be automatically eliminated when the others have been solved. As can be seen, the antigens available during the early attempts were soluble aqueous crude extracts of adult worms which could be obtained readily in sufficient quantity either from the patients or from animals experimentally infected with these flukes. The early studies on *C. sinensis* therefore used these buffered saline crude somatic extracts for intradermal tests, complement fixation assays, and precipitation reactions. It was subsequently recognized that these aqueous extracts were not suitable for the complement fixation test as they often exhibit anticomplementary activity, and the extraction procedure was therefore modified. Such an interference could be largely eliminated by the use of fat-free antigens which could be readily obtained by ether extraction prior to the preparation of the crude somatic extract. Interference in the complement-fixation test could be further minimized by partial purification through the use of ion-exchange chromatography and gelfiltration.

Like other intradermal skin tests, skin testing in clonorchiasis suffers from the same reasons, including standardization of antigen and subjective interpretation of the results. Moreover, the test using the various crude somatic extracts gave a considerable degree of cross reaction with other parasites including paragonimiasis and schistosomiasis.[21,25] The test therefore has been abandoned by most recent investigators.

Most of the previous attempts on immunodiagnosis for *C. sinensis* infection have been concentrated largely on complement fixation reactions. Although difficulties arising from anti-complementary activity of the antigens have been satisfactorily overcome, the difficulty with inherent cross reaction among different parasite still exists. The latter makes it rather difficult to interpret the results, particularly when people in endemic areas are often infected simultaneously by other intestinal or tissue parasites. This situation is even worse when the previous history or fecal specimens are not available for confirmation. Sawada *et al*[31] attempted to purify the crude somatic extract for CF test by passing the delipidated aqueous and sonicated extract through a Sephadex G-100 column and then passing the void volume fraction through ion-exchange columns. The final preparation, more than 90% carbohydrate and later identified to be polyglucose, gave a satisfactory result in the CF test. However, the specificity of this antigen preparation was never rigorously analyzed. On top of this, complement-fixation reactions also suffer from the lability of the reagent, difficulties in standardization and the tedious protocols, thus making procedures based on CF rather unpopular for diagnostic laboratories, particularly when more simple and accurate techniques are now available.

The use of precipitation reactions as in the gel diffusion technique have been studied by a few laboratories. The partially purified CF antigen of Sawada *et al*[31] failed to give a precipitin reaction with patient sera. Subsequently, Sun and Gibson[16] showed strong precipitation reactions using concentrated metabolic products of adult worms. These metabolic products contained both carbohydrates and proteins. Enzyme activities associated with digestion including amylase, invertase, maltase, lactase and esterase have been detected. Attempts to use egg antigens have been reported by Sun,[15] and Sun and Gibson.[17] While these investigators could not demonstrate by precipitin reaction the presence of anti-egg antibodies in the serum of patients and experimentally infected animals, egg components were nevertheless antigenic, as shown by the presence of antibodies in animals immunized with egg extract. We have also observed that serum from infected hamsters and from rabbits immunized with either adult crude extract or metabolic products reacted with eggs released by the worms *in vitro* (Unpublished observations).

Attempt has also been made to use an indirect hemagglutination test for the diagnosis of clonorchiasis.[32] These investigators used triethanolamine buffered saline extract of delipidated crude somatic antigen to coat tanned sheep red blood cells and found it to be fairly sensitive, using sera from rabbits experimentally infected with *C. sinensis*. However, its value in clinical diagnosis has not yet been evaluated.

Passive cutaneous anaphylaxis for the detection of reaginic antibody in rabbits experimentally infected with *C. sinensis* has given encouraging results.[21] These investigations, using borate-buffered saline extract of adult worms, showed the assay to be fairly sensitive in experimental animals. A positive reaction, suggestive of the presence of reagin or IgE antibodies, was observed in some animals within 3 weeks of infection. However, this test is impractical for the diagnosis of the disease. If the appearance of IgE antibody is expected, its detection by other means such as ELISA or RIA will be more practical, and this point should be investigated.

Very recently the use of ELISA for the diagnosis of human clonorchiasis has been reported in Chinese literature.[33] These investigators compared the reactivity of various aqueous crude somatic extracts, *i.e.*, triethanolamine buffered saline, veronal buffered saline and phosphate buffered saline, and showed all 3 to be quite satisfactory in distinguishing patients from normal healthy controls. Moreover, a fairly good correlation between ELISA values and worm burdens expressed as eggs per gram of feces was noted. Although not yet vigorously investigated, the antigens used by these investigators seem to be specific when tested against a limited number of sera from patients with other parasitic infections. It could not be determined from the data available as to the composition or complexity of these crude antigens.

In contrast to clonorchiasis, investigation on opisthorchiasis, particularly that caused by *O. viverrini* is more scanty. What information regarding the immunodiagnosis of opisthorchiasis is available pertains largely to *O. felinous*.[27-30] The two techniques used were the indirect hemagglutination reaction and ELISA. All work employed crude somatic extracts of adult *O. felineus*. The reports dealing with indirect hemagglutination showed that it was possible to diagnose acute cases of infection by this technique.[27,28] Antibody was detected in 53 of 54 patients with acute infection but it was positive in less than 50% of patients with chronic infection. However, many healthy controls also gave low antibody titer with this antigen. On the other hand, Ponomareva,[29] and Ponomareva and Alekseeva[30] comparing intradermal tests, indirect hemagglutination and ELISA using crude somatic extract of adult *O. felineus,* found ELISA to be the best, and results seemed to correlate satisfactorily with the intensity of infection. Using an ELISA technique, these investigators were able to distinguish acute from chronic infections. Patients with acute infection had high IgM ELISA titers and relatively low IgG ELISA titers. A reversed situation was true for those with chronic infection. However, there was no information available regarding specificity of the test.

In contrast to opisthorchiasis caused by *O. felineus,* only limited information is currently available on attempts at immunodiagnosis of opisthorchiasis caused by *O. viverrini*. Janechaiwat *et al*[34] were able to detect many bands of precipitation in the sera of patients infected with *O. viverrini* by immunoelectrophoresis. Sera from some patients showed as many as 5 bands with this antigenic extract. However, the test lacked sensitivity as less than 80% of the patients whose stools were positive for *O. viverrini* eggs gave positive results. The proportion of positive specimens decreased with decreasing intensity of infection. More serious difficulty was the fact that some sera from patients with other parasitic diseases including gnathostomiasis and schistosomiasis also reacted positively in this technique. Using indirect hemagglutination, we were able to detect agglutinating antibody against crude somatic extract in the sera from all hamsters infected with *O. viverrini* 30 days beforehand.[35] However, a proportion of these infected hamsters, particularly those with high level of infection exhibited some antibody within 15 days of infection. Because the antibody titers obtained by this technique were not very high, we felt that the test was not sensitive enough to be of clinical usefulness and therefore needed to be improved. Feldheim and Knobloch reported satisfactory results using ELISA tchnique.[24] Although they were able to distinguish the patients form uninfected controls, a considerable degree of cross-reaction was detected, including patients infected with *F. hepatica, P. africanus, P. uterobilaberalis* and *Schistosome* species. Attempt to use this technique to monitor the success of anthelminthic treatment gave equivocal results, as in about 50% of the cases, no significant reduction in antibody titer was noted in speimens taken 12 months after treatment. Using ELISA technique, we recently observed that while it was possible to distinguish patients with positive fecal egg counts from normal, the technique used could not clearly

distinguish these patients from those with other parasitic infection.[36] However, it was not possible to rule out the possibility that the latter group might have had previous contact with these flukes. Using an appropriate animal model would give a more clear cut answer to this question. A similar conclusion was reached also by other groups using ELISA[37] and immunofluorescent techniques.[19]

Current approaches to develop immunodiagnostic methods for opisthorchiasis

It is clear form these studies that if one is to have a more specific serological test for the diagnosis of parasitic infections, including those caused by liver flukes, one needs a more refined antigen for whatever test system is used to detect the specific antibody. However, before this can become a reality, one has to know fairly extensively the antigenic mosiac of the parasite under study. This, in turn, is possible only when the host-parasite relationship is more fully understood. In attempting to do this, we have now begun to characterize the various antigens from *O. viverrini* and have preliminary data suggesting the presence of specific parasite antigens which may have serodiagnostic value.[38] Once a specific antigen (s) is identified and characterized, one has to think about mass production. The latter has to be cheap as the disease occurs largely in developing countries. In this regard, it is likely that the genetic engineering approach could be used, since it has made possible the availability of several other biological reagents for medical use.

Last but not least, it is also possible to develop an immunodiagnostic technique for the detection of *O. viverrini* antigens in those suspected of being infected with the parasite. The most likely possibility is a specific reagent for the detection of eggs or other soluble products in the feces of these people. Here monoclonal antibodies could play a significant role. These various approaches will be employed by our group in an attempt to develop suitable immunodiagnostic methods which would be specific for infection caused by *O. viverrini*. If our attempt is successful, then it should be possible for other investigators to use a similar approach for infections caused by other parasities, *i.e., O. felineus* and *C. sinensis*.

Conclusion

Liver fluke infections caused by *Opisthorchis viverrini, O. felineus* and *Clonorchis sinensis* are still a major health problem in Southeast Asia, the Far East, and Central and Eastern Europe, with approximately 40-50 million people currently infected. Moreover, infections have been reported recently from several countries. Moreover, infections have been reported recently from several countries previously known to be free of the disease. The latter is attributed, at least in part, to ever increasing numbers of immigrants and refugees from countries in the endemic regions. Because of this increasing occurrence of the disease, clinicians and laboratory personnel in both endemic and non-endemic regions must be more aware of the possibility of opisthorchiasis and clonorchiasis in patients with gastrointestinal problems. It is for this reason that a simple, reliable, less-time consuming and more objective method for diagnosis is needed. Identification and large-scale production of specific antigen (s) should be initiated and used for the development of suitable immunodiagnostic methods for the detection of specific antibodies, either in the serum or other body fluids. The screening and production of monoclonal antibodies specific for parasite epitopes should be encouraged and made available for the detection of specific parasite antigen (s). If these various immunodiagnostic methods that are to be developed can be supplied in quantity cheaply, they would undoubtedly replace the more classical, time consuming and more subjective methods currently available. Such a development would also be an invaluable tool for epidemiology studies and for monitoring the outcome of anthelmintic treatment.

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Stitaya Sirisinha
Department of Microbiology Faculty of Science Mahidol University, Rama VI Road, Bangkok 10400, Thailand.

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