Exploring the interface between diagnostics and maps of neglected parasitic diseases

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SUMMARY

Although not new, the 'One Health' concept is gaining progressively more importance in parasitology. Now more than ever, veterinary and human perspectives should be closely joined in diagnosis and surveillance of neglected parasitic diseases. We argue that concerted, standardized and harmonized diagnostic and surveillance strategies are needed for the control and/or elimination of animal and human neglected parasitic infections. A key challenge is to integrate parasitological data with available geospatial methods in an accessible and user-friendly framework. We discuss the capability of new diagnostic devices (e.g. Mini-FLOTAC) and geospatial technologies supported by mobile- and electronic-based approaches as one of the research priorities of the new millennium.

Key words: neglected parasitic diseases, soil-transmitted helminths, gastrointestinal nematodes, One Health, copromicroscopic diagnosis, Mini-FLOTAC, field diagnosis, geospatial health.

INTRODUCTION

Neglected (from the Latin *neglegere* = to neglect; *nec* = not and *legere*: to select) means 'not treated with proper attention'. This term is used in human and veterinary medicine to indicate infections/diseases – mostly caused by parasites – often disregarded by the scientific community and health authorities with respect to their diagnosis and surveillance, but also research focus and/or funding.

From a public health perspective, the concept of neglected diseases usually refers to human tropical infections for which the name 'neglected tropical diseases' (NTDs) was coined in 2005 (thoroughly reviewed by Utzinger *et al*. 2012). Among the human NTDs, those caused by helminths predominate and, in particular, soil-transmitted helminths (STHs), i.e. *Ascaris lumbricoides, Trichuris trichiura* and hookworms (*Ancylostoma duodenale* and *Necator americanus*) are a major cause of malnutrition, anaemia and growth and mental delay (Bethony *et al*. 2006). STHs affect approximately 2 billion individuals worldwide and are a major public health issue in developing countries, predominantly in rural settings with inadequate sanitation especially in the poorest regions of Africa, Asia, Latin America and the Caribbean (Beaumier *et al*. 2013). However, the impact of these infections is not confined to tropical settings. Globalization and global movement of people have brought STHs and other human NTDs to the attention of health systems in many 'developed' countries, particularly among travellers, expatriates and migrants (Utzinger *et al*. 2012); as an example, a recent study showed the presence of STHs in about 5% of immigrants in Naples, southern Italy (Gualdieri *et al*. 2011).

From a veterinarian perspective, among parasitic infections of livestock, gastrointestinal nematodes (GIN), caused by different genera of helminths (e.g. *Ostertagia, Teladorsagia, Haemonchus, Trichostrongylus, Cooperia, Nematodirus*, etc.) still represent a significant economic and welfare burden to the global ruminant livestock industry (Morgan *et al*. 2013). However, as described above for human STHs, GIN infections in grazing ruminants are often neglected and implementation of research, diagnosis and surveillance of these parasites is still poor, mainly in the matter of standardized diagnostic methods and geographical information system (GIS)-based surveillance.

Auspiciously, matters are improving and more attention has been paid in the last few decades toward human and veterinary neglected parasitic infections. For example, over the past 30 years, increased recognition of the impact of the helminth burden on human health has led to an expansion of a number of large-scale control and elimination initiatives against these NTDs (Boatin *et al*. 2012). Concerning animal health, the ranking of GIN as one of the top causes of lost productivity in ruminants by the recent DISCONTOOLS programme (http://www.discontools.eu/home/index) witnesses the EU's increasing consideration of the impact of these parasites upon animal health, welfare and productivity (Vercruysse, present issue).

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Veterinary and human perspectives should be united in the diagnosis and surveillance of neglected parasitic infections. Although not new, the 'One Health' concept – which states that 'there should be a seamless interaction between veterinary and human medicine with clinicians, researchers, agencies and governments working together' (Day, 2011) – is gaining more importance in parasitology. The 'One Health' perspective entails a strategy that addresses events at the intersection of human, domestic animal, wildlife and ecosystem health (Zhou, 2012) and close collaboration between veterinarians and tropical disease specialists would improve intervention measures (Demeler *et al*. 2013). Hence, in the following sections we emphasize the need for concerted diagnostic and surveillance strategies for the control and/or elimination of animal and human neglected parasitic infections. We also underline that the urgent priority of the new millennium is to accelerate the development and evaluation of novel (and/or standardization of already existing) diagnostics together with harmonized mapping and monitoring systems through GIS and other geospatial tools.

ON DIAGNOSTICS AND DIAGNOSIS

Diagnosis of parasitic infections is of pivotal importance for both individual patient management and population-based studies, such as drug efficacy trials and surveillance of control programmes, in both human and veterinary public health (Cringoli *et al*. 2010).

The general principles put forth by the TDR Diagnostics Tests for infectious diseases (Banoo *et al*. 2010) indicate that 'To be useful, diagnostic methods must be accurate, simple and affordable for the population for which they are intended. They must also provide a result in time to institute effective control measures, particularly treatment'. Both the performance characteristics (e.g. sensitivity, specificity, reproducibility, positive predictive value, negative predictive value) and the operational features (e.g. simplicity, ease of use, user acceptability) should be considered whenever a diagnostic test is evaluated (Solomon *et al*. 2012).

Regarding human NTDs, the paper by Becker *et al*. (2013) has recently provided a comprehensive overview of the reference diagnostic tests (based on microscopy, culture, immunology and molecular biology) presently available for helminths (including STHs) and other infectious pathogens causing persistent digestive disorders in the tropics. Regarding the diagnosis of neglected veterinary parasitic infections, the same approaches (microscopic-/cultural-/immunologic-/molecular-based) described in Becker *et al*. (2013) can be utilized.

In the present era of genomics, metagenomics, proteomics and bioinformatics (Roeber *et al*. 2013), diagnosis of STHs in human and GIN in animals still relies predominantly on copromicroscopy (Cringoli *et al*. 2010; Demeler *et al*. 2013) and analysis of faecal samples for the presence of helminth eggs is the most widely used diagnostic procedure both in veterinary and human parasitology. Since its foundation by C. J. Davaine in 1857, several copromicroscopic techniques have been developed, in particular quantitative methods based on faecal egg counts (FEC) to determine infection intensity through the assessment of eggs per gram of faeces (EPG).

FEC techniques are considered relatively straightforward and protocols such as the McMaster technique and the Wisconsin flotation technique in the veterinary field, and the Kato-Katz technique and the ether-based concentration method in the human field have been available (and have remained unchanged) for many years (Cringoli *et al*. 2010). However, each of these copromicroscopic techniques shows strengths and limitations. Furthermore, they vary considerably according to their performance and operational characteristics (e.g. analytic sensitivity, accuracy and precision in assessing FEC, timing and ease of use). While copromicroscopy is considered as highly specific, its sensitivity depends on the intensity of infection (Utzinger *et al*. 2012) and also on the detection limit of the employed technique. The recently introduced use of FLOTAC techniques (Cringoli *et al*. 2010) now theoretically allows sensitivities as low as 1 EPG, i.e. 24 times more than with a single Kato-Katz thick smear and 50 times more than with a single McMaster slide. Several studies have demonstrated that the FLOTAC technique outperformed multiple Kato-Katz thick smears and McMaster for the diagnosis and drug efficacy assessment of STH infections in different settings (e.g. Utzinger *et al*. 2008; Knopp *et al*. 2009, 2011; Glinz *et al*. 2010; Albonico *et al*. 2013). Furthermore, FLOTAC outperformed McMaster and Wisconsin techniques for the diagnosis and drug efficacy assessment of GIN in ruminants (Levecke *et al*. 2011, 2012; Rinaldi *et al*. 2011).

However, a main limitation of FLOTAC is the complexity of the technique, which involves centrifugation of the sample with a specific device, equipment that is often not available in all laboratories, especially in developing countries (Levecke *et al*. 2012). To overcome these limitations, under the 'FLOTAC strategy' (Fig. 1) of improving the quality of copromicroscopic diagnosis, a new simplified tool has been developed, i.e. the Mini-FLOTAC, having an analytic sensitivity of 10 EPG (Cringoli *et al*. 2013). It is an easy-to-use and low-cost method, which does not require any expensive equipment or energy source, and can be used to perform FEC in animals and humans (Cringoli *et al*. 2013). It is recommended that Mini-FLOTAC (Fig. 1) be used in combination with Fill-FLOTAC (Fig. 2), a disposable sampling kit, which consists of a container, a

Fig. 1. Devices of the 'FLOTAC Family': Mini-FLOTAC, FLOTAC and Fill-FLOTAC.

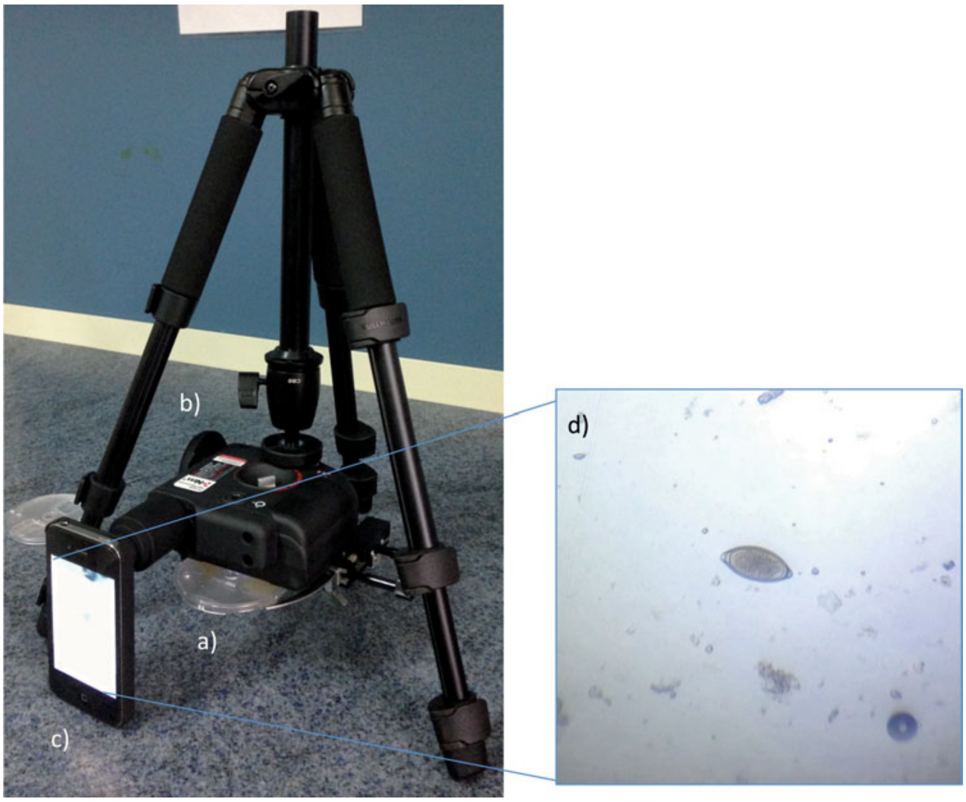

Fig. 2. Mini-FLOTAC (a) under the Newton NM1 compact portable microscope (note the inverted position) (b). *Trichuris* egg (d) visualized by a mobile phone (c) adapted to the portable microscope.

collector and a filter. Fill-FLOTAC facilitates the performance of the first four consecutive steps of the Mini-FLOTAC technique, i.e. sample collection and weighing, homogenization, filtration and filling (Cringoli *et al*. 2013).

Mini-FLOTAC has been already validated in veterinary parasitology for the diagnosis of helminths (e.g. ascarids, hookworms, trichurids, gastrointestinal nematodes) in pets and livestock (Cringoli *et al*. 2013; Rinaldi *et al*. in press). Concerning human parasitic infections, recent experiences from the field in India and Tanzania suggested that Mini-FLOTAC is a valid, sensitive and potentially low-cost alternative technique that could be used in resource-limited settings – particularly for STH diagnosis (Barda *et al.* 2013*a*, *b*, *c*). The advantages of Mini-FLOTAC compared with Kato-Katz, which is the WHO standard recommended technique, is that it can be performed both on fresh and preserved stool samples. This will allow the samples to be processed in subsequent days from their collection with a more efficient quality control. Detailed tutorials of the Mini-FLOTAC techniques on fresh and fixed faeces can be found on the following websites: http://www.youtube.com/watch?v=61C_bpBPbyg (Mini-FLOTAC technique–fresh faeces) and http://www.youtube.com/watch?v=hjMn6mepv_s (Mini-FLOTAC technique–fixed faeces).

It is our hope that further validations of Mini-FLOTAC for diagnosis of helminths parasitizing both animals and humans in different epidemiological settings will demonstrate that this new device meets the standards of a quality-ASSURED (affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free, deliverable) diagnostic test.

LIMITATIONS AND FUTURE OF COPROMICROSCOPIC TECHNIQUES

Although widely used for diagnosis of intestinal parasites, it is well known that copromicroscopy is prone to a number of shortcomings (Utzinger *et al.* 2012).

First, there is a clear lack of standardization of copromicroscopic techniques and usually each lab uses ‘its own’ method mostly based on the ‘lab traditions’ rather than on the performance or operational characteristics of the technique. However, it is important to underline that different factors may influence the performance of any copromicroscopic technique, especially those based on flotation (e.g. McMaster, Wisconsin, FLOTAC and Mini-FLOTAC) and sedimentation. These can include the choice of fixative used for faecal preservation, the duration of faecal preservation before analyses, the selection of the flotation solution, the concurrent use of ether and many other factors. Therefore, interlaboratory standardization of techniques, as well as internal and external quality control for parasitological data, are strongly required in human and veterinary parasitology.

Second, the results of any copromicroscopic technique strongly depend on the accuracy of laboratory procedures but also on the experience of the laboratory technicians reading the microscopic fields (Becker *et al.* 2013). Hence, the reliable identification of parasitic infections requires in-depth training for specimen preparation, and expertise and experience for subsequent microscopic examination (Utzinger *et al.* 2012). Therefore, the ‘human’ factor (i.e. the hands and eyes of technicians) is of fundamental importance for copromicroscopic analyses compared with other diagnostic approaches (i.e. immunological or molecular methods).

Third and most importantly, the main limitation of copromicroscopy is the time and cost to conduct copromicrocopic analysis (in particular FEC) on a representative number of individuals. However, this limitation can be overcome by performing FEC on pooled samples, in which equal amounts of faeces from several individuals are mixed together and a single FEC is used as an index of group mean FEC (Morgan *et al.* 2005). Recently, such pooling approaches have been applied to fresh stool samples using the McMaster technique and to fixed faecal samples using the chlorazol black dye for the detection of intestinal parasites in humans and results indicated that this is an efficient and potentially cost-effective strategy (Gaafar, 2011; Mekonnen *et al.* 2013). Similarly, also in the veterinary field, pooling sheep faecal samples using Mini-FLOTAC has been demonstrated as a rapid procedure that holds promise as a valid strategy for assessing GIN infections in ruminants (Rinaldi *et al.* in press).

Together with pooling, one of the challenges of the future of copromicroscopy is to perform diagnosis of neglected parasitic infections directly in the field by using field portable kits including the new generation of field microscopes. This aspect has had a long track record of development with prior microscope models. As an example, Stothard *et al.* (2005) reported a field evaluation of a handheld microscope for diagnosis of intestinal schistosomiasis in Ugandan schoolchildren. This handheld microscope was suggested as a pragmatic alternative to the compound microscope, playing an important role in the collection of prevalence data to better guide anthelmintic drug delivery and also empowering the diagnostic capacity of peripheral health centres where compound microscopes are few or absent (Stothard *et al.* 2005). More recently, Bogoch *et al.* (2013) described the proof of concept that a mobile phone can be converted into a microscope for the point-of-care diagnosis of STHs in resource-constrained settings. It is therefore evident that using portable field microscopes without the need for electricity would be the optimal solution for the diagnosis of helminths in health periphery (for STH) and on farms (for GIN). A closed diagnostic device such as Mini-FLOTAC could be easily ‘attachable’ to such kinds of microscopes supplied with adaptors for mobile phone camera (Fig. 2).

ON MAPPING AND GEOSPATIAL TOOLS

There is increasing consensus that standardized diagnostic techniques should be linked to geospatial tools (e.g. GIS, remote sensing, virtual globes, spatial statistics, ecological niche models) for the integrated mapping, monitoring and surveillance of neglected parasitic infections in humans and animals (Solomon *et al.* 2012; Cringoli *et al.* 2013). Now more than ever, we can use the old adage that a picture is worth more than a thousand words (Utzinger *et al.* 2011). Presentation of parasitological results based on interactive, computer-generated maps represents a straightforward way of visualizing large numbers of datasets in a geographical context that could have different spatial scales (local, national, regional and global) (Bergquist and Tanner, 2012; Cringoli *et al.* 2013). Geospatial tools have now become an integral part of epidemiology and surveillance by driving systematic collection, analysis and interpretation of health data (Malone and Bergquist, 2012; Zhou *et al.* 2013). Translational research has rapidly evolved from approaches requiring specialists in GIS and remote sensing technology to a level where it has become a normal part of health planning and implementation of national disease control programmes (Malone and Bergquist, 2012).

Regarding human NTDs, the paper by Clements *et al.* (2013) has recently provided a comprehensive overview of the geospatial instruments (along with the application, advantages and disadvantages of each of them) relevant to achieve malaria elimination. The same geospatial approaches can be translated to other neglected parasitic infections of public health and veterinary relevance such as STHs and GIN.

Representation of epidemiological data in the form of a map facilitates interpretation, synthesis and recognition of any changing frequency and pattern of infected cases and the appearance of clusters of parasitological phenomena. Moreover, maps are a convenient tool to foster discussion and dialogue among different stakeholders (Clements *et al.* 2013; Cringoli *et al.* 2013). However, the use of GIS and other geospatial tools for disease mapping, does by no means overcome the major concern of any empirical research, namely (parasitological) data quality. It is noteworthy to stress that disease maps are indissolubly intertwined with diagnostics and further research on geospatial technology and links with standardized diagnostic techniques must take place (Cringoli *et al.* 2013). The use of different diagnostics (with differing sensitivity, negative predictive value and/or other performance characteristics) gives rise to distribution maps that can change substantially. As an example, we used the data from our paper by Gualdieri *et al.* (2011) where 514 immigrants to Naples (southern Italy) were tested comparing the FLOTAC and the ethyl acetate concentration techniques; combined results of the two techniques served as a diagnostic 'gold standard'. Regarding STHs, eggs of *T. trichiura* were found in 20 (3·9%) immigrants when examined by FLOTAC and only in 2 (0·4%) subjects when examined by the concentration technique (Gualdieri *et al.* 2011). By using this dataset, combined with GIS, the parasitological data were geo-referenced and two distribution maps for *T. trichiura* were obtained, using the results obtained by FLOTAC (Fig. 3) and by the ethyl acetate concentration (Fig. 4) techniques. From the analysis of these data, it is evident that the map in Fig. 4 is a biased sub-set of the 'true' *T. trichiura* distribution showed in Fig. 3.

Therefore, a key challenge for human and animal neglected parasitic infections is to integrate routinely collected data with available geospatial methods in an accessible and user-friendly framework (Clements *et al.* 2013; Morgan *et al.* 2013). Compared with the past decades, the situation is improving with the development of open-source software for GISs (e.g. QGIS), online data warehouses containing freely available geospatial data and increased training opportunities in geospatial sciences.

Furthermore, the ability to define the distribution of infections at regional, national and sub-national levels has been enhanced greatly by the increased availability of good quality survey data combined with the use of sophisticated model-based geostatistics, enabling spatial prediction in unsampled locations (Magalhães *et al.* 2011). Hence, Bayesian geostatistical models are increasingly being used in human and veterinary epidemiological studies to estimate parasitic risk profiling at high spatial resolution (e.g. Magalhães *et al.* 2011; Catelan *et al.* 2012; Chammartin *et al.* 2013; Scholte *et al.* 2013).

Also, WebGIS tools have become increasingly popular as a result of advances in computer technologies, improved and established geographical standards which have helped the dissemination of spatial data to different audiences, and the shift from expert tools to community-based tools that are accessible to a wider range of users (Kienberger *et al.* 2013). Closely related to (simple) web-based GIS tools are spatial decision support systems (SDSS), i.e. interactive GIS-based platforms (including integrated database management systems) that are designed to support place-based decision making at the various stages of a planning process (Duncombe *et al.* 2012).

In order to stimulate the exchange of information on application of geospatial tools in health research through publications, international conferences, workshops and training courses, GnosisGIS (www.gnosisgis.org) was founded in 2000 as 'Network On Snail-borne Infections with special reference to Schistosomiasis (GnosisGIS)'. The initial aim was to develop and consolidate collaboration dedicated to validation and use of GIS and other geospatial tools to control schistosomiasis and other snail-borne infections. However, its scope has since been expanded to include other infectious and parasitic diseases of medical and veterinary importance. In 2005 the acronym GnosisGIS was kept, but the network was renamed 'Global Network for Geospatial Health'. In August 2013, the name was officially changed to 'International Society for Geospatial Health–GnosisGIS'. It is a dynamic, multinational and

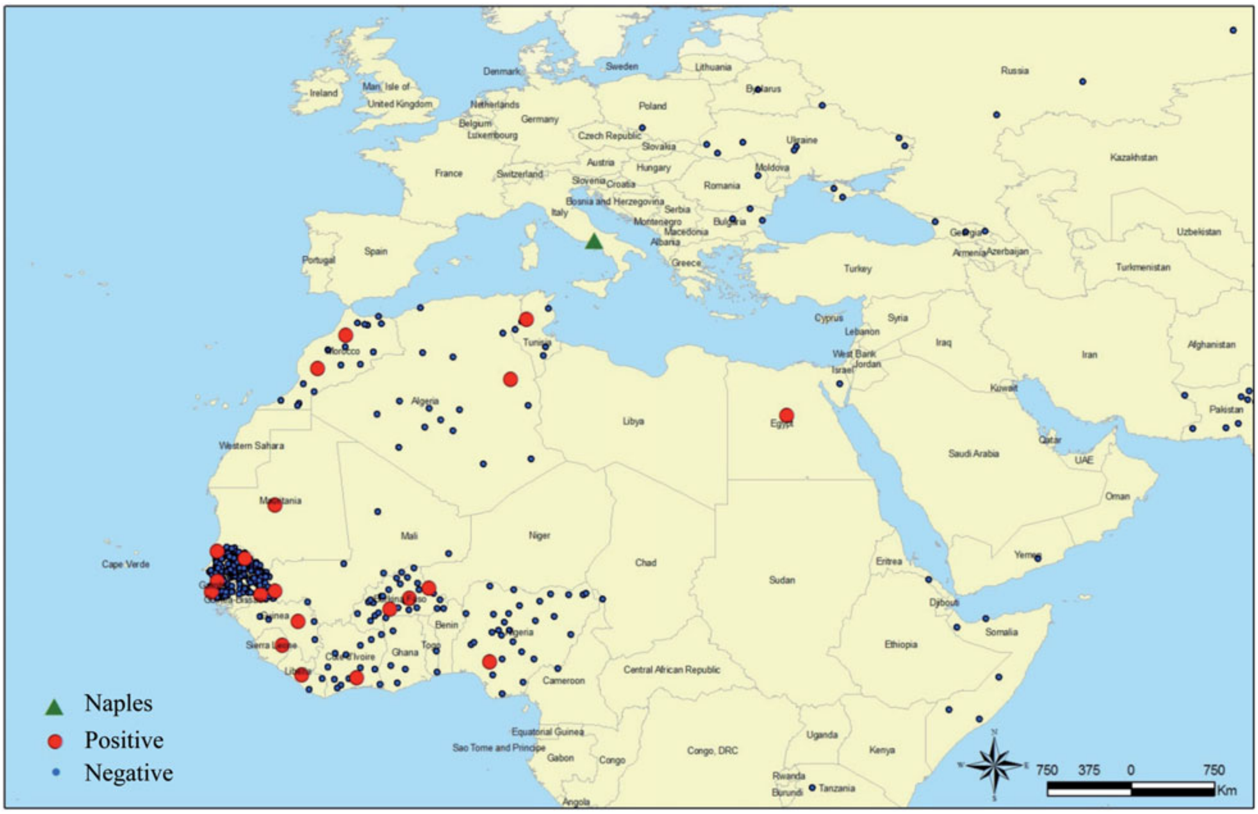

Fig. 3. Map of positivity to *Trichuris trichiura* by FLOTAC. Geo-referenced parasitological results of immigrants tested for intestinal parasites in Naples, southern Italy (data from Gualdieri *et al*. 2011).

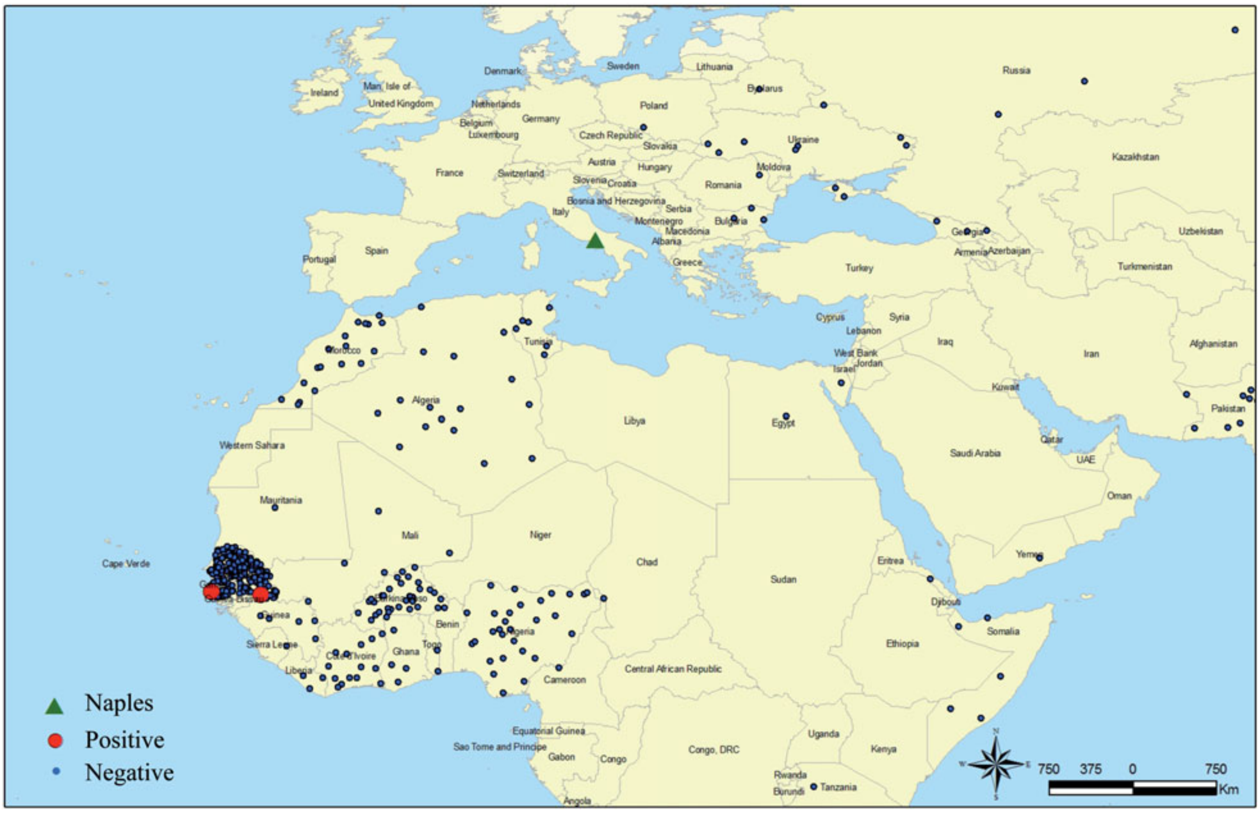

Fig. 4. Map of positivity to *Trichuris trichiura* by the ethyl acetate technique. Geo-referenced parasitological results of immigrants tested for intestinal parasites in Naples, southern Italy (data from Gualdieri *et al*. 2011).

multidisciplinary scientific society, bringing together veterinarians, medical doctors, biologists, parasitologists, climatologists, geographers, computer scientists and statisticians. GnosisGIS strives to advance the use of geospatial tools in veterinary and public health. Toward this end, one of the main products of GnosisGIS is its official journal, *Geospatial Health*, an international, peer-reviewed scientific journal, founded in 2006 (Utzinger *et al*. 2011). Recently, as a unique feature of *Geospatial Health*, a new section predominantly based on visual presentations, including brief video clips, was introduced in 2010 under the banner of 'vHealth' (visual health) (Bergquist and Tanner, 2012). We are convinced that the visual approach will facilitate communication with the wide variety of stakeholders involved in research today, e.g. government offices, funding agencies, etc. However, we should keep in mind that working with policy makers and stakeholders to use the outputs of geospatial analysis in a way that helps their ability to make improved evidence-based decisions will be a very complex challenge (Clements *et al*. 2013).

CONCLUSIONS AND PERSPECTIVES

There is a broad political commitment towards large-scale control and progressive elimination of a wide variety of parasitic diseases; however, there are some worrying signs for the future, particularly the anticipated declines in funding and coverage of key interventions, and the paucity of novel diagnostic tools and surveillance strategies (Cringoli *et al*. 2013). Importantly, 'improve existing/develop novel diagnostic test', and 'update and share data platforms to optimize data management, analysis and modelling (using geospatial technologies), integrating scientists, stake holders and end-users' are among the priorities identified by the TDR reference group on helminth infections (Boatin *et al*. 2012). Furthermore, for the upcoming calls of Horizon 2020 research funding, transdisciplinary research is strongly recommended by the European Commission. We have already emphasized the need for integrating sound epidemiological designs with innovative diagnostic tools and strategies and high-resolution geospatial tools for mapping neglected parasitic infections of animal and humans considering the 'One-Health' perspective (Cringoli *et al*. 2013). Recognizing these challenges, standardization of quality procedures and innovating, validating and applying new tools and strategies will foster and sustain long-term control of neglected parasitic infections of human and animals. Indeed, effective and timely public health responses depend upon the ability of health systems to provide accurate and timely information for action (Zhou *et al*. 2013). Use of new technologies supported by mobile- and electronic-based (m- and e-health) approaches as well as improved and more sensitive strategies of diagnosis is considered one of the research priorities to strengthen the surveillance systems within national control/elimination programmes of NTDs (Zhou *et al*. 2013), including neglected parasitic infections of animals and humans.

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