Preliminary Evidence for the Absence of Cystic Echinococcosis in Gabon: A Cross-Sectional Pilot Survey in Humans and Definitive Hosts

Felix Lötsch,^{1,2} Ghyslaine Mombo-Ngoma,² Johannes Mischlinger,^{1,2} Mirjam Groger,^{1,2} Luzia Veletzky,^{1,2} Ayôla Akim Adegnika,^{2,3} Bertrand Lell,^{2,3} Selidji Todagbe Agnandji,^{2,3} Marielle Bouyou-Akoté,⁴ Markus Obermüller,¹ Marion Wassermann,⁵ Renate Schneider,⁶ Herbert Auer,⁶ and Michael Ramharter^{1,2,3,7*}

¹Division of Infectious Diseases and Tropical Medicine, Department of Medicine I, Medical University of Vienna, Vienna, Austria; ²Centre de Recherches Médicales de Lambaréné, Lambaréné, Gabon; ³Institut für Tropenmedizin, Universität Tübingen, Tübingen, Germany; ⁴Département de Parasitologie-Mycologie Médecineropicale, Faculté de Médecine, Université des Sciences de la Santé, Libreville, Gabon; ⁵Department of Parasitology, University of Hohenheim, Stuttgart, Germany; ⁶Department of Medical Parasitology, Institute of Specific Prophylaxis and Tropical Medicine, Center of Pathophysiology, Infectiology and Immunology, Medical University Vienna, Vienna, Austria; ⁷Department of Tropical Medicine, Bernhard Nocht Institute for Tropical Medicine & I. Department of Medicine, University Medical Centre, Hamburg-Eppendorf, Hamburg, Germany

Abstract. Cystic echinococcosis (CE) is a globally endemic zoonosis caused by the larval stage of the *Echinococcus granulosus* sensu lato (s.l.) complex. Although the disease is known to be highly prevalent in certain parts of North and East Africa, data on CE, both in humans and definitive hosts, are extremely scarce for Central Africa. The present study assessed the epidemiology of CE in humans and dogs in rural Gabon. An ultrasound and serologic survey was conducted in volunteers from rural villages in Gabon. A two-step approach was used for serological testing with an indirect hemagglutination assay as a screening test and Western Blot as a confirmatory test. Fecal dog samples were analyzed microscopically, and polymerase chain reaction (PCR) amplification of *nad1* and *cox1* genes was performed when taeniid eggs were visible. Regional hospitals and the national reference center for parasitology in Gabon were contacted for information about previous cases of CE. Randomly selected communities were invited to participate. Three hundred and forty-eight human volunteers from these communities were screened. No suspected cases of CE were detected. Definitive host screening was performed from 128 fecal samples from representative subregions, but no eggs from *E. granulosus* s.l. were found. No documented cases of echinococcosis were reported from the local health-care institutions and the national diagnostic reference center in Gabon. Cystic echinococcosis seems to be very rare or absent in Gabon. The reason for this lack of evidence for echinococcosis is unknown, but the absence of livestock may play a major role.

INTRODUCTION

Cystic echinococcosis (CE) is a global zoonosis caused by the larval stage of the *Echinococcus granulosus* sensu lato (s.l.) complex—the dog tapeworm. Carnivores, especially dogs, are the definitive hosts, livestock the intermediate, and humans the aberrant intermediate hosts. In intermediate hosts (and humans), the ingestion of *E. granulosus* eggs can cause the subsequent development of a cyst in an internal organ—most frequently the liver or the lung. The metacestode—the cyst—then starts to produce protoscolices, of which each can develop into an adult worm when ingested by a definitive host. The cysts cause the morbidity and mortality associated with this parasitosis.

In several parts of Africa, morbidity due to CE is high and a broad spectrum of genotypes causing CE has been documented.^{1–3} *Echinococcus granulosus* is highly endemic in dogs in North Africa and certain areas of East Africa. Previous studies have shown a prevalence of 25.8% of *Echinococcus* spp. eggs in stray dogs in Libya and 33–39.4% in stray dogs in the Turkana District of Kenya.^{4–6} However, data from the Central African region are extremely rare. In a coproscopic survey of dogs in northeastern Gabon (Ogoué-Ivindo), taeniid eggs were identified in 8.6% of the samples. However, it was not further determined whether these eggs were *Echinococcus* spp. or *Taenia* spp.⁷ In another study from Libreville, the capital of Gabon, taeniid eggs were found in only 1% of canine stool samples.⁸

To our knowledge, there are no systematic studies available investigating human echinococcosis in Central Africa. The geographically closest reports, retrospective analyses of hospital records, and one serological survey stem from Nigeria in West Africa, reporting prevalences of 0% and 0.53%.^{9,10} Both studies were performed in the 1980s. One case report of a patient from the Central African Republic has been published, in which molecular characterization revealed the *Echinococcus canadensis* G6 strain.¹¹ In addition, a patient with a splenic cyst caused by *E. granulosus* has been reported from Yaoundé, Cameroon,¹² a case in which a refugee from Niger has been reported from Italy,¹³ and an older case report documented a case in a male European living in DR Congo.¹⁴ Besides these case reports, no systematic data are available from the Central African region.

Consequently, epidemiological surveys of human echinococcosis in Central Africa are required. Extensive epidemiological surveys and knowledge about the distribution, transmission, life cycles, ecosystems, pathogenicity, and prevalence of *E. granulosus* s.l. are crucial for prevention programs and eradication purposes. Thus, the aim of this pilot study was to investigate the presence of CE in central Gabon and describe first epidemiologic features of this parasitosis in the Central African ecosystem.

METHODS

Ethics and regulatory approval. Ethical clearance for this study was sought from the institutional ethics committee of the Center de Recherches Médicales de Lambaréné (CER-MEL): No. 006/2013. Regulatory approval was received from the “Centre National de la Recherche Scientifique et Technologique”: AR0004/14/ and AR0005/16.

* Address correspondence to Michael Ramharter, Bernhard Nocht Hospital for Tropical Diseases, Bernhard Nocht Institute for Tropical Medicine and University Medical Center Hamburg-Eppendorf, Hamburg 20246, Germany. E-mail: ramharter@bnitm.de

Study site and study period. The study was conducted in Central African Gabon and coordinated at the CERMEL, a nongovernmental research institution situated in the province of Moyen-Ogooué.¹⁵ Details of the study area are depicted in Figure 1. The study region lacks large-scale breeding of live-stock. However, goats are commonly held as livestock by villagers, and there is a large number of stray dogs throughout the country besides domestic dogs, which are also used for hunting purposes. The climate is equatorial and a large proportion of the country is covered by tropical rainforest.¹⁶ The rainy season lasts from October to May (with a short dry season from December to January) with up to 500 mm of rainfall per month. June through August are months of the dry season with little rainfall.

Infectious diseases account for the largest proportion of health-care consultations in the study area¹⁷ and the burden of parasitic diseases is high.^{18,19} The ultrasound survey and serologic analyses were conducted from January to September 2014 and canine fecal samples were sampled in April and May 2016.

Ultrasound-based and serologic survey in humans. In the absence of any firm evidence to estimate the prevalence of

CE, no formal sample size calculation was performed. Based on logistical resources and ethical considerations, a sample size of 350 participants was targeted for this first pilot study. To decrease the risk of selection bias, communities in the study area were randomized, using an online tool (www.random.org/lists) and then visited by the research team in the respective sequence. If access to the village was not possible, no community representatives were available, or participation was refused, the research team moved on to the next village on the list. All villagers aged 18 years or older were invited to participate.

Investigators provided information to the community representatives. In these sessions, the purpose of the study and the disease and its transmission cycles were discussed, and all issues with regard to screening, potential findings, and subsequent patient care were explained. Based on in-depth discussions with community representatives, the willingness and feasibility of screening in the respective community were assessed. In case of assent by the community representatives, a time schedule was agreed on to perform ultrasound and serological screening. On days of screening, all community members fulfilling the inclusion criteria were invited to

FIGURE 1. Map of the study area. Stool samples were collected at places indicated with stars and on connecting roads in between. The coordinating center was located in Lambaréné as indicated with a yellow star. Humans were screened in the province of Moyen-Ogooué (shaded in blue with red borders). Hospitals that were contacted for previous cases and the reference center for parasitology are indicated by an arrow. This figure appears in color at www.ajtmh.org.

participate in the survey. Each participant was informed about the study, study procedures, potential risks associated with the study, issues of patient care, and participants' rights in case of consent to the study. Written informed consent was sought from all participants before any study procedure. Blood samples (2 × 9 mL) were drawn for screening on specific antibodies against *E. granulosus*. Tests used were indirect hemagglutination assay (IHA—Celognost Echinococcosis, Siemens®, Marburg, Germany), and Western Blot (WB—Echinococcus Western Blot, LDBIO Diagnostics®, Lyon, France) to confirm positive IHA results. For ultrasonography, a portable Mindray® DP10 device (Shenzhen, China) with a curved array transducer was used. Abdomen ultrasound focusing on the evaluation of potential liver cysts is a proven, rapid, and reliable screening tool for CE.²⁰ A trained physician performed systematic liver-focused abdominal ultrasonography in the villages. In addition, a questionnaire was completed by all participants to evaluate possible risk factors for CE including agricultural activity, owning of dogs, cultivation of vegetables, eating of wild fruits, and owning a garden.

In addition, attending physicians of two local hospitals disposing of an ultrasound machine and the reference center for parasitology in the capital were contacted and asked about previous cases of CE. The reference center for parasitology is the only of its kind in the country.

Coprosopic survey in dogs. For the coprosopic survey, a haphazard sampling approach was chosen with the goal to cover a wide geographic area of Gabon. Spontaneously excreted fecal samples from dogs were collected using a 30-mL coprotainer and cooled for transport to the research facility. Then, samples were stored at −80°C for at least 10 days to eliminate infectivity of parasitic eggs and reduce hazards for study staff. For isolation of taeniid eggs, approximately 2 cm³ of each sample were concentrated using a flotation technique based on a zinc chloride solution. Details have been published elsewhere.^{21,22} The samples were then analyzed microscopically and taeniid-like eggs were separated individually for molecular analysis. Nested PCR with primers for both mitochondrial *nad1* and *cox1* genes was performed on individual eggs.²³ Exact confidence intervals (CIs) were calculated for proportions of patients and samples positive for CE using STATA 14.0 (StataCorp., College Station, TX) for Mac. In case of zero observations, a one-sided 95% CI is presented. Image 1 (map) was created using QGIS for Mac and is based on OpenStreetMap.

No survey in intermediate hosts was performed as there was no single official abattoir operable at the time of the study.

RESULTS

Host survey in humans. In total, 348 individuals from 17 communities participated in the ultrasound survey. The median age was 56 years (IQR: 43–71.5 years). One hundred and ninety-nine (57.2%) were female and 149 (42.8%) male. In none of the participants, cystic lesions suspicious for CE could be documented. Other incidental findings such as gallstones or solitary kidney cysts were common but were not further analyzed.

Serum samples were taken from 347 participants; in one participant, venipuncture failed. Eight patients (2.3%) tested positive at low titers (max. 1:128) in the hemagglutination assay, but all samples were negative in the confirmatory WB.

Thus, 0/348 (0%; one-sided 95% CI: 0–0.86%) were considered to have CE.

Five of these eight patients were further evaluated for other parasitic infections by stool and urine samples. *Ascaris* eggs were found in two samples, *Trichuris* eggs in two samples, hookworm eggs in two samples, and *Strongyloides* in one sample. None of these patients had *Schistosoma* eggs. Behavioral and demographic risk factors are presented in Table 1.

Definitive host survey. Screening of dog feces for *Echinococcus* spp. was performed in all subregions of Gabon. In total, 128 samples were collected and further processed. Taeniid-like eggs were identified microscopically in three samples (2.3%). All three samples were tested negative in both PCR protocols. In summary, no eggs of *E. granulosus* s.l. were detected in fecal samples of the definitive hosts (0%; one-sided 95% CI: 0–2.31%). Sampling sites are indicated in Figure 1.

Survey of hospitals and the national reference center for medical parasitology. Attending physicians at two hospitals in the study region were contacted to retrieve information about suspected or confirmed cases of human echinococcosis. The Albert Schweitzer Hospital is located in the province of Moyen-Ogooué and the Regional Hospital of Fougamou is located in the neighboring province of Ngounié. In addition, the reference center for medical parasitology in the capital Libreville was also contacted. No case of CE had been documented in any of these institutions.

DISCUSSION

The present study was the first to systematically investigate the presence of CE in Central African Gabon, and the results may be used representative for neighboring countries with similar ecosystems as data on CE in the Central African region are currently extremely scarce.³ The World Health Organization considers human echinococcosis as one of 17 neglected tropical diseases and attributes the status “suspected” to Gabon with regard to human echinococcosis,²⁴ although it is unclear on which data this assessment is based.

In this study, no case of CE was detected, neither in humans nor in dogs—the most important definitive host. So far, no clinical cases of CE have been documented in regional hospitals or the reference center for parasitology. The dog fecal sampling survey may be considered as the most reliable epidemiological assessment as it has the highest sensitivity to detect echinococcosis transmission. The absence of *E. granulosus* in these fecal samples collected in all subregions of the country

TABLE 1
Behavioral and demographic risk factors of human volunteers participating in the ultrasound survey

Risk factor	n (%)
Agricultural activity	286/348 (82.2)
Cultivating vegetables	276/348 (79.3)
Own garden	14/348 (4.0)
Owning a dog	161/348 (46.3)
Hunting	70/348 (20.1)
Eating wild fruits	347/348 (99.7)
Activities in the forest	264/348 (75.9)

therefore provides important evidence for the absence of CE in this Central African region.

One single case of CE from a neighboring country was reported from Yaoundé, Cameroon.¹² However, the climate and ecosystem in Cameroon, especially in the north of the country, is much drier than that in Gabon, and larger numbers of livestock, especially cattle, are present. This indicates that CE is (very) rare or absent in the tropical rainforest ecosystem of Gabon. One possible reason is that important intermediate hosts such as cattle or sheep are rarely kept in Gabon. Only goats, which are less suitable hosts for *E. granulosus* s.l., are commonly found in close proximity to humans in the study region. It may only be speculated whether sylvatic life cycles with wild definitive and intermediate hosts exist in the Central African ecosystem, as evidence for this is currently lacking. Behavioral differences of humans in Gabon compared with regions with high incidences could be another reason, although this seems unlikely, given that several classic risk factors for CE are prevalent in Gabon. A large proportion of villagers were found to be involved in agricultural activities; usually subsistence farming or keeping dogs is common in rural regions.

Also, when examining fecal samples of dogs as the most important definitive hosts, surprisingly, only three samples contained taeniid eggs (eggs of *Taenia* spp. and *Echinococcus* spp. are microscopically not distinguishable). However, this aligns well with the two other studies from Gabon that reported on taeniid eggs in canine stool samples. In a coproscopic survey of dogs in northeastern Gabon (Ogoué-lvindo), taeniid eggs were identified in 8.6% of the samples.⁷ In another study from Libreville, the capital of Gabon, taeniid eggs were found in 1% of canine fecal samples only.⁸

Several limitations of this study should be addressed. First of all, the sample size in the ultrasound survey may have been too low to detect CE as a rare disease. Nevertheless, the methodologically sound selection process of communities and participants guaranteed high representativeness of the data for this Central African region. Second, only the liver was screened during the survey. Although the liver is by far the most commonly encountered site of hydatid cysts, other organs, especially the lung—which is more difficult to assess by ultrasound—may be affected as well. However, the addition of serological testing increased the sensitivity of this epidemiological survey. Third, some of the canine fecal samples might have been from other animals or humans. To minimize the risk, the samples were collected either after direct observation or with the help from locals from open public places, which seemed inappropriate for human use. A random sampling method was used for this pilot study. However, it should also be mentioned that CE usually is not evenly distributed but occurs in pockets where certain risk factors are present in combination. One of these risk factors is large-scale livestock breeding (e.g., of cattle), which is basically absent in Gabon. Thus, we could not identify areas with a high a priori suitability for CE for a more targeted screening.

Future studies should survey intermediate host animals for the presence of cystic lesions at both formal and informal slaughterhouses and bush meat markets. Also, the systematic assessment of patient records at more hospitals at a countrywide level disposing of imaging modalities such as ultrasound, computed tomography (CT), and magnetic resonance imaging (MRI) is probably warranted for a more solid conclusion on the presence or absence of CE in Gabon.

Received December 7, 2017. Accepted for publication February 11, 2018.

Published online May 21, 2018.

Acknowledgments: We are grateful for the support of CENAREST (Centre National de la recherche scientifique) in Gabon. We would like to thank the participants for their cooperation and the field workers, especially Ms. Cécile Bendoume, for their tremendous support and contribution to the implementation of this study.

Financial support: We are grateful for the financial support of the Landsteiner Gesellschaft, Austria.

Authors' addresses: Felix Lötsch, Mirjam Groger, and Markus Obermüller, Department of Infectious Diseases and Tropical Medicine, Medizinische Universität Wien, Wien, Austria, E-mails: felix.loetsch@gmail.com, mirjam.groger@gmail.com, and markus.obermueller@meduniwien.ac.at. Ghyslain Mombongo-Ngoma, Centre de Recherches Médicales de Lambaréné, Albert Schweitzer Hospital, Lambaréné, Gabon, E-mail: ghyslain.mombongoma@gmail.com. Johannes Mischlinger and Luzia Veletzky, Centre de Recherches Médicales de Lambaréné, Lambaréné, Gabon, E-mails: hannes.mischlinger@gmail.com and luzia.veletzky@meduniwien.ac.at. Ayôla Akim Adegnika, Centre de Recherches Médicales de Lambaréné, Lambaréné, Gabon, and Institute of Tropical Medicine, University Hospital Tübingen, Tübingen, Germany, E-mail: aadegnika@gmail.com. Bertrand Lell, Centre de Recherches Médicales de Lambaréné, Lambaréné, Gabon, E-mail: bertrand.lell@gmail.com. Selidji Todagbe Agnandji, Centre de Recherches Médicales de Lambaréné, Lambaréné, Moyen-Ogooué, Gabon, E-mail: agnandjis@lambarene.org. Marielle Bouyou-Akote, Department of Parasitology Mycology, Université des Sciences de la Santé, Libreville, Gabon, and Malaria Clinical Research and Operational Unit, Centre Hospitalier de Libreville, Libreville, Gabon, E-mail: mariellebouyou@gmail.com. Marion Wassermann, Department of Zoology, Universität Hohenheim, Stuttgart, Baden-Württemberg, Germany, E-mail: marion.wassermann@uni-hohenheim.de. Renate Schneider and Herbert Auer, Department of Medical Parasitology, Institute of Specific Prophylaxis and Tropical Medicine, Vienna, Austria, E-mails: renae.schneider@meduniwien.ac.at and herbert.auer@meduniwien.ac.at. Michael Ramharter, Division of Infectious Diseases and Tropical Medicine, Department of Medicine I, Medical University of Vienna, Vienna, Austria, and Institute of Tropical Medicine, University of Tübingen, Tübingen, Germany, and Bernhard Nocht Hospital for Tropical Diseases, Bernhard Nocht Institute for Tropical Medicine and University Medical Center Hamburg-Eppendorf, E-mail: ramharter@bniitm.de.

REFERENCES

1. Wahlers K, Menezes CN, Wong ML, Zeyhle E, Ahmed ME, Ocaido M, Stijns C, Romig T, Kern P, Grobusch MP, 2012. Cystic echinococcosis in sub-Saharan Africa. *Lancet Infect Dis* 12: 871–880.
2. Romig T et al., 2011. Echinococcosis in sub-Saharan Africa: emerging complexity. *Vet Parasitol* 181: 43–47.
3. Deplazes P et al., 2017. Global distribution of alveolar and cystic echinococcosis. *Adv Parasitol* 95: 315–493.
4. Buishi I, Njoroge E, Zeyhle E, Rogan M, Craig P, 2006. Canine echinococcosis in Turkana (north-western Kenya): a coproantigen survey in the previous hydatid-control area and an analysis of risk factors. *Ann Trop Med Parasitol* 100: 601–610.
5. Buishi IE, Njoroge EM, Bouamra O, Craig PS, 2005. Canine echinococcosis in northwest Libya: assessment of coproantigen ELISA, and a survey of infection with analysis of risk factors. *Vet Parasitol* 130: 223–232.
6. Macpherson CN, French CM, Stevenson P, Karstad L, Arundel JH, 1985. Hydatid disease in the Turkana District of Kenya, IV. The prevalence of *Echinococcus granulosus* infections in dogs, and observations on the role of the dog in the lifestyle of the Turkana. *Ann Trop Med Parasitol* 79: 51–61.
7. Normand T, Bourry O, Dang H, Leroy E, Bourdoiseau G, Davoust B, 2006. Enquête sur le parasitisme digestif des chiens dans une zone rurale du Gabon. *Bull. Acad. Vét. France* 159: 59–68.
8. Beugnet F, Edderaï D, 1998. Epidemiological survey on digestive and blood helminths of dogs in Libreville, Gabon. *Rev Med Vet (Toulouse)* 149: 327–330.

9. Sixl W, Rosegger H, Schneeweiss H, Withalm H, Schuhmann G, 1987. Serological investigations in Nigeria for anthroponoses in human sera: brucellosis, echinococcosis, toxoplasmosis, chlamydial diseases, listeriosis, rickettsiosis (*Coxiella burneti* and *Rickettsia conorii*). *J Hyg Epidemiol Microbiol Immunol* 31 (4 Suppl): 493–495.
10. Dada BJ, 1980. Taeniasis, cysticercosis and echinococcosis/hydatidosis in Nigeria: I—prevalence of human taeniasis, cysticercosis and hydatidosis based on a retrospective analysis of hospital records. *J Helminthol* 54: 281–286.
11. Develoux M, Enache-Angoulvant A, Gounant V, Brian E, Khalil A, Bazelly B, Hennequin C, 2011. Hepatic and pulmonary cystic echinococcosis in a patient from the Central African Republic. *Travel Med Infect Dis* 9: 88–90.
12. Firmin AA, Bernadette NN, Catherine M, Roger D, Eric T, Oudou N, Claude NNE, McManus DP, Eimo M, 2013. Intracystic bleeding of a solitary hydatid cyst: a rare complication of a rare disease in central Africa. A case report. *Case Rep Clin Med* 2: 163.
13. Angheben A, Mariconti M, Degani M, Gobbo M, Palvarini L, Gobbi F, Brunetti E, Tamarozzi F, 2017. Is there echinococcosis in West Africa? A refugee from Niger with a liver cyst. *Parasit Vectors* 10: 232.
14. De Meulemeester J, Dardenne G, 1958. Hydatid cyst of the liver in a European living in the Belgian Congo. *Acta Chir Belg* 57:283–295.
15. Ramharter M et al., 2007. History and perspectives of medical research at the Albert Schweitzer Hospital in Lambaréné, Gabon. *Wien Klin Wochenschr* 119 (19–20 Suppl 3): 8–12.
16. The World Factbook—Central Intelligence Agency. Gabon. Available at: <https://www.cia.gov/library/publications/the-world-factbook/geos/gb.html>. Accessed November 28, 2017.
17. Manego RZ et al., 2017. Demography, maternal health and the epidemiology of malaria and other major infectious diseases in the rural department Tsamba-Magotsi, Ngounie Province, in central African Gabon. *BMC Public Health* 17: 130.
18. Lötsch F, Obermüller M, Mischlinger J, Mombo-Ngoma G, Groger M, Adegnika AA, Agnandji ST, Schneider R, Auer H, Ramharter M, 2016. Seroprevalence of *Toxocara* spp. in a rural population in central African Gabon. *Parasitol Int* 65: 632–634.
19. Adegnika AA, Ramharter M, Agnandji ST, Ateba-Ngoa U, Issifou S, Yazdanbakhsh M, Kremsner PG, 2010. Epidemiology of parasitic co-infections during pregnancy in Lambaréné, Gabon. *Trop Med Int Health* 15: 1204–1209.
20. Brunetti E, Kern P, Vuitton DA; Writing Panel for the WHO-IWGE, 2010. Expert consensus for the diagnosis and treatment of cystic and alveolar echinococcosis in humans. *Acta Trop* 114: 1–16.
21. Wassermann M, Aschenborn O, Aschenborn J, Mackenstedt U, Romig T, 2015. A sylvatic lifecycle of *Echinococcus equinus* in the Etosha National Park, Namibia. *Int J Parasitol Parasites Wildl* 4: 97–103.
22. Mathis A, Deplazes P, Eckert J, 1996. An improved test system for PCR-based specific detection of *Echinococcus multilocularis* eggs. *J Helminthol* 70: 219–222.
23. Hüttner M, Nakao M, Wassermann T, Siefert L, Boomker JDF, Dinkel A, Sako Y, Mackenstedt U, Romig T, Ito A, 2008. Genetic characterization and phylogenetic position of *Echinococcus felidis* (Cestoda: Taeniidae) from the African lion. *Int J Parasitol* 38: 861–868.
24. Echinococcosis—Epidemiology, World Health Organization. Available at: <http://www.who.int/echinococcosis/epidemiology/en/>. Accessed March 12, 2018.