APPLICATION OF THE ENZYME-LINKED IMMUNOELECTROTRANSFER BLOT TO FILTER PAPER BLOOD SPOTS TO ESTIMATE SEROPREVALENCE OF CYSTICERCOSIS IN BOLIVIA

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Abstract. An enzyme-linked immunoelectrotransfer blot (EITB) assay was used to study the prevalence of cysticercosis in rural Bolivia. Dried blood spots on filter paper from fingersticks were used as assay samples. Before the serosurvey, experiments were performed to show that samples eluted from dried whole blood on filter paper exhibited no decrease in sensitivity when compared with the more traditional serum samples used in the EITB. Fingerstick blood dried on filter paper is a convenient, economical way of transporting and storing field samples for epidemiologic surveys of cysticercosis in developing countries. This report shows the utility of this sample collection method in underdeveloped countries where refrigeration is not possible and where venipuncture is a problem. Blood was obtained from randomly selected residents in three rural regions of Bolivia: Chuquisaca (n = 1,859), Cochabamba (n = 1,516), and Tarija (n = 1,010). The estimated seroprevalence on 10% of the sample collected for the three regions were 9%, 4.5%, and 2%, respectively.

Cysticercosis is caused by infection of tissues by the larval stages of Taenia solium. The disease is clinically diverse because cysts can occur in virtually any anatomic location. Neurocysticercosis is the most commonly recognized manifestation. In addition to taeniasis, an intestinal infection by the adult tapeworm, cysticercosis is endemic throughout the developing world where swine husbandry practices and human consumption of inadequately cooked pork favor transmission. In South America, neurocysticercosis is a major cause of morbidity creating the loss of huge amounts of resources every year.1 Recent studies in Peru and Mexico, for example, have demonstrated seroprevalences ranging from 8% to 12%, and have confirmed that neurocysticercosis is a major cause of neurologic morbidity in those countries.2-7

In Bolivia, unpublished data from surveys performed in the altiplano region indicate that the seroprevalence of cysticercosis in some communities ranges from 10% to 19%. Observed pork consumption and pig husbandry practices in these regions suggest that taeniasis/cysticercosis disease may be a significant, but largely unrecognized, public health problem. In this study, we examined the seroprevalence of cysticercosis in the Bolivian regions of Tarija, Chuquisaca, and Cochabamba.

In addition to estimating the seroprevalence of human cysticercosis in Bolivia, this study evaluated blood collection in the form of dried blood spots on filter paper and found that many of the problems of collecting, processing, and transporting blood samples for serologic surveys can be overcome by the use of small volumes of blood collected by finger stick and dried onto filter paper. This simple technique negates difficulties in cold-chain transport of serum or plasma as well as cultural problems associated with venipuncture. Various filter paper methods have been in use for many years for other assays.8-11 The present report shows the utility of this sample collection method in an enzyme-linked immunoelectrotransfer blot (EITB) assay for cysticercosis.

MATERIALS AND METHODS

Filter paper samples preparation. All samples were collected by finger stick and spotted onto Whatman (Clifton, NJ) #1 filter paper. The filter papers were air-dried and stored at ambient temperature until use. For testing, 7.0-mm disks were punched out of the center of each dried blood spot and placed into 500 μl of phosphate-buffered saline/Tween 20/azide (0.10 M NaCl, 0.05 M Na2PO4, pH 7.2, with 0.3% [v/v] Tween 20 and 0.1% [w/v] Na3C03). Samples were sonicated (Waterbath Sonicator Model #8-12; Branson, Shelton, CT) for 15 min continuously. Eluted samples can be stored until needed after sonication in PBS/Tween 20/azide at −20°C. The volume of dried blood on a 7.0-mm disk of filter paper was approximately equal to 3.5 μl of liquid whole blood.

Patient study. All samples used in this study were part of an infectious disease survey sample archive collected in the early 1990s under the aegis of the Bolivian Ministry of Health, in accordance with its human subjects policies. All samples were tested in the present study with no patient identification. Fingerstick blood samples were obtained from 1,859 residents from the region of Chuquisaca, 1,010 residents from Tarija, and 1,516 from Cochabamba. Ten percent of these samples were randomly selected from each of the three regions (yielding 186 samples from Chuquisaca, 101 from Tarija, and 158 from Cochabamba), and tested for antibodies to cysticercosis.

The EITB using filter paper samples. The EITB assay for T. solium-specific antibodies was performed as previously described.12 Briefly, seven lentil-lectin purified T. solium glycoprotein antigens were used in an EITB format to detect infection-specific antibodies eluted from the whole blood spots. Each sonicated eluent was diluted with an equal volume of PBS/Tween 20/10% nonfat dry milk and mixed well before use as the antibody source. Antibody reactions against these glycoproteins were visualized with the H2O2/diaminob-
FIGURE 1. Comparison of the sensitivities between filter paper blood and liquid whole blood. The enzyme-linked immunoelectrotransfer blot was prepared by exposing resolved antigen strips to a serially diluted *Taenia solium*-positive serum in liquid whole blood and dried blood extracted from filter paper. The approximate volume equivalence of dried blood to liquid whole blood is 3.5 μl. After serum and conjugate exposure, strips are developed with 3,3’ diaminobenzidine.

enzidine substrate system. An antibody reaction to one or more glycoprotein bands was considered a positive result.

RESULTS

Technique optimization. Before applying this method to the Bolivian serosurvey, it was optimized by using a laboratory spiked whole blood sample. A positive control serum was formed by pooling equal volumes of serum from seven persons with parasitologically confirmed cysticercosis. This positive control serum was serially diluted using normal human whole blood as the diluent. The whole blood used as the diluent was collected from U.S. residents who had no risk from cysticercosis and were EITB negative. This spiked whole blood titration was dried onto filter paper and processed as described above. Comparing a titration of liquid and filter paper blood using the EITB assay demonstrated that dried blood spots can be eluted from filter paper by sonication and used as the antibody source for the cysticercosis EITB assay. In the assay, there were minimal differences in sensitivity between dried blood spots and liquid whole blood as shown in Figure 1.

Seroprevalence. The seroprevalences for Chuquisaca, Cochabamba, and Tarija were 9%, 4.5%, and 2%, respectively. In Chuquisaca, 16 of 186 (seven males and nine females) were EITB-positive. In Cochabamba, of 158 total samples, two males and five females were confirmed as EITB positive. In Tarija, the only two EITB-positive samples were males. These results are summarized in Figure 2.

DISCUSSION

Previous studies have estimated the prevalence of cysticercosis to be between 10% and 19% in some communities of the altiplano region of Bolivia. Other studies in Mexico and Peru, which demonstrated similar prevalence of cysticercosis, confirmed that neurocysticercosis is the leading cause of neurologic morbidity in these areas. Seroprevalence in the three regions examined in this study ranged from 2% in Tarija to 9% in Chuquisaca. Although the seroprevalence was lower in the survey areas than previous estimates, these types of surveys identify areas that would benefit from intervention strategies and eradication efforts.

Many of the problems of collecting, processing, and transporting blood samples for serologic surveys and the problems associated with venipuncture in developing countries can be overcome by the collection of small amounts of blood by fingerstick. In addition, this is a rapid and relatively inexpensive way to sample large populations in rural areas of developing countries. This study demonstrates that dried whole blood collected on filter paper can be successfully used in the EITB assay for cysticercosis. The serum samples collected by this method was found comparable to serum obtained from liquid whole blood without any decrease in assay sensitivity. The conveniences of acquiring blood samples using filter paper far outweigh the extra time involved in processing samples after they arrive in the laboratory.
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