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USE OF SENTINEL PIGS TO MONITOR ENVIRONMENTAL *TAENIA SOLIUM* CONTAMINATION

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Abstract. We tested a novel approach to assay *Taenia solium* prevalence using the enzyme-linked immunoelectrotransfer blot assay in sentinel piglets to determine environmental contamination with *T. solium* eggs in a disease-endemic zone in Peru. Twelve sentinel piglets from an area where the disease is not present were tested at two months of age, moved to an area where the disease is endemic, and retested at the of age nine months. Sentinel piglets native from this *T. solium*–endemic area were also tested concurrently at two and nine months of age. Of the non-native pigs, 33% (4 of 12) acquired new infection. Of the 28 native pigs tested, 64% (18 of 28) acquired the infection. In a subset of the native piglets from seronegative sows, 44% (4 of 9) were infected at five months of age. Serodiagnosis of sentinel piglets is a practical method to detect *T. solium* eggs in the environment. Furthermore, it permits indirect assessment of human risk, which may be useful for monitoring the efficacy of intervention programs.

Human cysticercosis is a common cause of sickness and death in developing countries,[1,2] and is attributed to 10–12% of hospital admissions for neurologic disease in disease-endemic areas.[3,4] Only humans can acquire adult tapeworms after ingesting cysts from infested pork. Eggs are shed into the environment via human feces and when ingested by either pigs or humans, develop into tissue cysts (causing cysticercosis).[5] The disease often attacks the human nervous system, causing a variety of neurologic symptoms, most commonly epilepsy.[3,4]

Most cysticercosis intervention programs use antiparasitic treatment, stool examination, and human serodiagnosis to determine disease prevalence, but these methods are generally expensive, slow, and difficult to comply with, partly because of cultural problems associated with obtaining human blood and stool samples.[6,7] Direct detection of eggs in the environment is extremely difficult because *Taenia* species eggs are scarce and large amounts of soil must be processed and examined microscopically to find a single egg.[6] Since pigs become infected only by ingesting eggs from human feces, pig infection rates must, therefore, reflect the relative amount of *Taenia solium* eggs in the environment. Obtaining blood samples from pigs is acceptable to villagers and is easily performed; thus, serodiagnosis on pigs may be a valid and practical way to monitor the potential for cysticercosis infection and can be used to evaluate the efficacy of control programs. A similar use of sentinel hamsters and mice to indicate schistosome contamination of the environment has been previously described.[8]

The enzyme-linked immunoelectrotransfer blot assay (EITB) is a sensitive and specific indicator of cysticercosis in pigs.[9] Using serum

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EITB, we tested two sentinel pig models. First, we placed non-native, uninfected piglets into a *T. solium*–endemic village and monitored seroconversion; then, we monitored seroconversion rates in native piglets. A subset of native piglets from sows of known EITB status was also evaluated.

MATERIALS AND METHODS

Twelve two-month-old piglets from Lima, Peru, a nonendemic area for the disease, were tested by serum EITB for *T. solium* antibodies and relocated to Maceda, an endemic area. All native two-month-old piglets in Maceda (n = 157) were also tested by EITB at the same time. All 12 non-native pigs and 28 surviving native pigs were retested at nine months of age. The sows of 115 of the native piglets were also tested, and these piglets were evaluated at five and nine months of age. All pigs were free-ranging.

Study site. Maceda, population 421, is a village in the Peruvian jungle that is highly endemic for cysticercosis, with a human seroprevalence rate in 1988 of 8%.[10]

Diagnostic serology. Infection status was determined using EITB serologic assay, performed as previously described.[11] This assay uses purified glycoprotein antigens (LL-GP) in an EITB format to detect infection-specific antibodies in serum. Peroxidase-labeled goat anti-swine antibody (Kirkegaard and Perry, Gaithersburg, MD) were used. Bound antibodies were visualized using an H_2O_2/diaminobenzidine (DAB) substrate system.[12] The EITB was interpreted with reference to reaction to seven glycoprotein bands commonly recognized by serum antibodies from infected human and swine. These bands are GP50, GP42-39, GP24, GP21, GP18, GP14, and GP13.[11,13] Reaction to any of these bands indicated *T. solium* infection in the non-native sentinel pigs and native piglets from seronegative sows. In piglets from infected (EITB+) sows, reactions to bands different from those of the mother were presumed to indicate new infection. Infection-specific antibody band patterns in the piglets are indistinguishable from maternally transferred antibody reactions. In contrast, new reaction bands produced by the piglet alone most certainly represents a new antigenic stimulus, and therefore, presumed to be the result of new infection.

Data analysis. The chi-square test and the Fisher's exact test were used to test associations between categorical variables.

TABLE 1

*Enzyme-linked immunoelectrotransfer blot (EITB) assay conversion rates in non-native and native piglets at nine months of age**

Population (type of piglets)	Month 2 EITB status, No. = n	Month 9 ETIB-positive, No. (%)	Month 9 With new EITB bands, No. (%)
Non-native	Negative (n = 12)	4 (33)	NA
Native†	Negative (n = 16)	9 (56)	NA
	Positive (n = 12)	NA	9 (75)

* NA = not applicable.
† The 28 native pigs are classified according to their initial (month 2) EITB status.

RESULTS

Sentinel piglets. All 12 of the non-native piglets survived the duration of the experiment (nine months). Only 28 of the original 157 native piglets surveyed initially at two months of age were surviving at nine months of age. The low survival rate is partially caused by natural death and the fact that pigs are usually slaughtered when they are a year old. In Peruvian villages, animal vaccination is not routine, and mortality varies greatly, depending on current infectious diseases. In Maceda, a hog cholera outbreak killed a large number of the pigs during our study period. Of the 12 non-native pigs, four specimens (33%) had acquired antibody to EITB bands after nine months, but these bands were rather faint. Of 28 native pigs, 18 (64%) acquired the infection by nine months of age; 75% (9 of 12) of initially positive native pigs showed new antibody bands and 56.2% (9 of 16) of the initially negative pigs showed new antibody bands. Although not statistically significant (P = 0.07), there was a strong trend for higher infection rates in native pigs (Table 1).

Piglets from sows of known EITB status. Of the native piglets from EITB+ sows, 23.8% (5 of 21) and 50% (4 of 8) were infected at five and nine months, respectively. At five months, 44% (4 of 9) of the piglets of EITB- sows were also infected (Table 2).

DISCUSSION

The sentinel pig model is a practical, novel approach for detecting *T. solium* eggs in the environment that will provide a practical means of

TABLE 2

Enzyme immunoelectrotransfer blot (EITB) assay conversion rates in native piglets from sows with known EITB status

Parent sow EITB status	Five months of age Total: 30 piglets		Nine months of age Total: 9 piglets	
	EITB+	EITB−	EITB+	EITB−
EITB+	5 (24%)*	16 (76%)	4 (50%)	4 (50%)
EITB−	4 (44%)	5 (56%)	1 (100%)	0

* All animals had EITB reaction band patterns different from those of their sows.

monitoring the efficacy of intervention programs. Three years before this experiment, mass niclosamide chemotherapy had been given to 93% of the seropositive persons in the study village of Maceda. At that time, 43% (57 of 153) of all pigs were EITB+.[8] The present study found 64% infection in a new cohort of sentinel native piglets by EITB. These results show that there is still environmental contamination with *T. solium* eggs, and that niclosamide, as applied, did not break the cycle of infection and transmission. Because pig populations are renewed yearly,[14] EITB positivity rates in piglets less than one year old will allow assessment of interventions and intensity of environmental contamination by *T. solium* with time.

This study compared native and non-native sentinel pigs and found important differences. First, non-native pigs have a trend toward lower infection rates, and their positive reactions are faint. This may be caused by a lower infection burden secondary to differences in feeding habits, genetic susceptibility, or humoral immune regulation.[13] Using the same model (i.e., native versus non-native), it may be possible to monitor an area over time. The advantage of a native pig model is that it appears to be more sensitive, since these animals convert at a higher rate, as well as practical, because it is hardier, requires no transportation, and is less expensive. Imported sentinel pigs, despite vaccination received prior to placement in the villages, rarely survived more than 1.5 years. Many deaths occurred when the imported hogs became pregnant. In utero adaptation to multiple environmental factors may also contribute to the better survival rate of native piglets. Since cohort sizes shrink rapidly because of slaughter and disease, shorter evaluation periods may be best. Determination of the optimum age of piglets to sample requires further study.

We must acknowledge that native piglet studies may be confounded in animals up to eight months of age by the persistence of maternal antibodies to *T. solium* antigens (unpublished data). However, this can be controlled by knowing the EITB status of the mother. Sows' EITB status may be easily determined in the field by testing their serum with EITB. Possible options to control for the effects of maternal antibodies are to select piglets from EITB-sows, or to retest piglets for acquisition of new reaction bands. Distinct reaction bands produced by the piglet alone and not the sow most certainly represent new antigenic stimulus, and therefore, presumed to be the result of an infection.

Testing of pigs offers a simple way to measure the efficacy of control programs, with minimal community participation. Thus, pigs can be a useful indicator and even a target for control programs.

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