TESTING OF PARASITES AND CHEMICAL RESIDUES IN A SLAUGHTERHOUSE

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SUMMARY

A sensitive and convenient high volume enzyme immunoassay test system has been developed and applied for screening of trichina and sulfamethazine in hogs. This system, optimised for slaughterhouse applications, is also applicable for screening of other pathogens, antibiotics and pesticides. Introduction of such a system in slaughterhouses for food animals will help in better management of carcasses and ensure the supply of pathogen and chemical safe meat to consumers.

INTRODUCTION

Surveillance of the parasite, *Trichinella spiralis*, has been undertaken in a hog slaughterhouse, but the procedures used to detect trichina, such as trichinascopic examination and pepsin-HCl digestion, are inadequate to fulfill the need of a modern hog slaughterhouse due to lengthy procedure and slow turnaround time (Ruitenberg et al. 1983). In addition to sporadic infection of trichina, the pork industry is facing the problem of chemical residues in finished meat products. The Food and Drug Administration has set a 0.1 ppm violative level for the sulfonamide antibiotic residues in pork tissues (U.S. Code Fed. Reg. 1983). However, the sulfamethazine violations, the key antibiotic used in raising hogs, are running 4-13% of all slaughtered animals and have not declined in the past several years, due in part to the lack of a suitable monitoring system (Williams 1984).

We report here the usefulness of a semi-automated enzyme immunoassay (EIA) monitoring system for trichina and sulfamethazine which is suitable to keep up with the kill speed of thousands of animals per hour in a commercial slaughterhouse.

EXPERIMENTAL

High Volume EIA for Trichina and Sulfamethazine. This is a microtiter plate based indirect EIA which uses trichina specific excretory antigen and goat anti-porcine antibody conjugated to alkaline phosphatase (Oliver et al. 1986). The animals entering the kill line are stunned and bled. The blood samples are collected into EDTA bar-coded tubes. Bar-code identification on the tubes are entered into a computer system with a bar-code wand. The tubes are then placed on a Tecan RSP 530 sample handling system in a disposable rack capable of holding the same number of tubes as the number of wells in the microtiter plates. The sample handler dilutes and distributes samples and reagents into the reaction wells of microtitration plates. After incubation and washing steps, the substrate is added. The absorbance optical densities (OD) are obtained and compared with the known positive and negative reference samples assayed on the same plate. Results of unknown samples are calculated as a percent of these standards.

$$\%EIA = \frac{\text{Sample OD - Neg. Ref. OD}}{\text{Pos. Ref. OD - Neg. Ref OD}} \times 100$$

Samples with values greater than 7% EIA are considered positive. The positives can be highlighted on the monitor, and the report printed.

Diaphragms were taken from each pig and analyzed for trichina by the pooled digestion technique (Zimmermann 1967).

The above Tecan system has been adapted to perform a high volume competitive EIA for sulfamethazine in plasma/serum of hogs which requires anti-sulfamethazine antibody coated microtiter wells and sulfamethazine-horseradish peroxidase conjugate (Singh et al. 1988). Percent binding is calculated from the absorbance in the absence (B_o) and presence (B) of sulfamethazine in standard/samples as (B/B_o) x 100. A standard curve is prepared by plotting log sulfamethazine vs. % binding or logit % binding.

Table 1. Recovery of Sulfamethazine Spiked in Plasma at Various Concentrations by EIA

sulfamethazine ug/mL added	recovered	% recovery
0.016	0.018	112.5
0.031	0.032	103.2
0.063	0.067	106.3
0.125	0.122	97.6
0.250	0.251	100.4
0.500	0.500	100.0

RESULTS

Trichina.

Of the 20,978 animals tested in a North Carolina slaughterhouse, 99.9% were negative by digestion method and had a mean EIA of -4.0%. Three hundred thirty-four mature breeding animals from herds with known trichinosis infections were also tested. These animals, of which 19% were positive by the digestion, had a mean %EIA of 26% (Fig.1).

Based on digestion results and %EIA for several thousand hogs, an EIA cut-off level of 7% was established. At this cut-off level, about 1.4% of the 20,978 animals tested positive for trichina antibody at the slaughterhouse. Comparison of testing by EIA with the tissue digestion method is given in Fig. 2.

Chemical Residue (Sulfamethazine).

The linear dose response curve was obtained by plotting logit % binding against sulfamethazine concentration (inset Fig. 3).

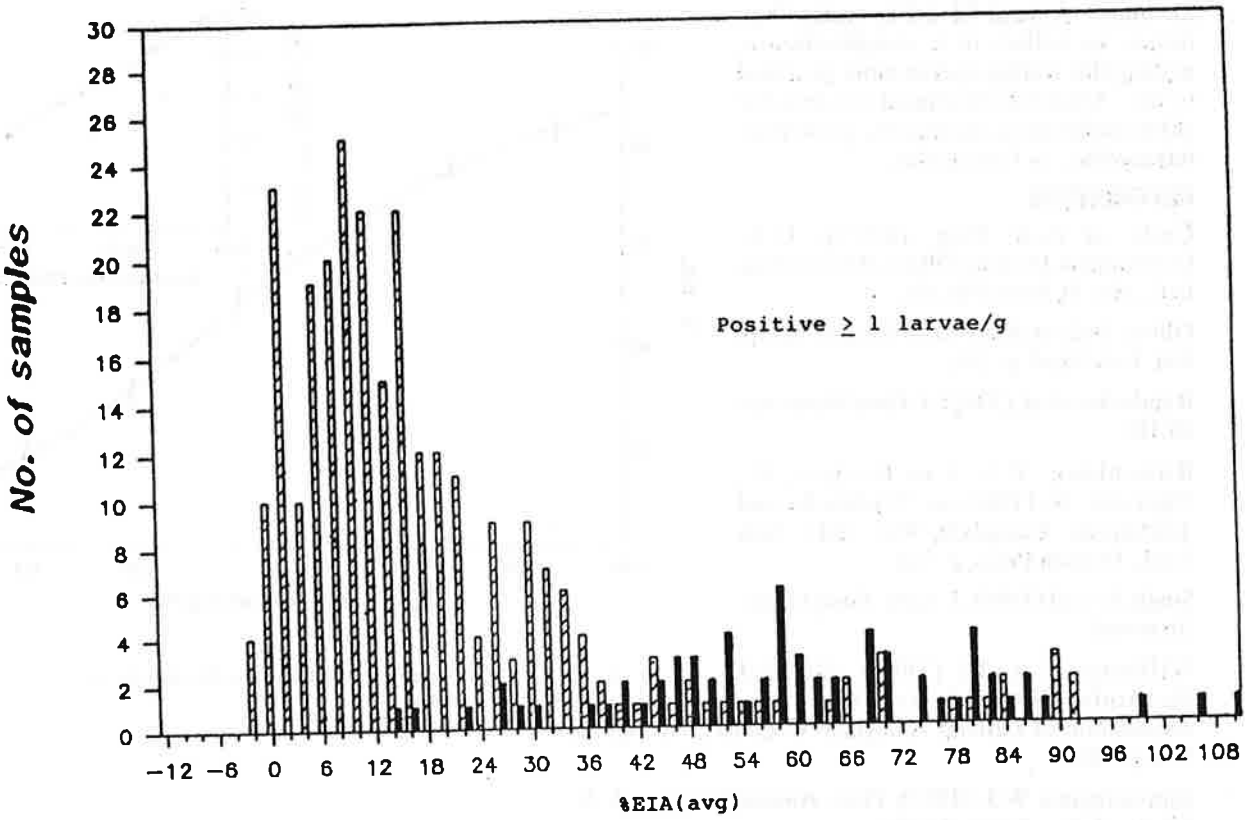

FIG. 1 EIA serology results on farm animals from known endemic areas. Samples supplied by USDA, Beltsville, Maryland.

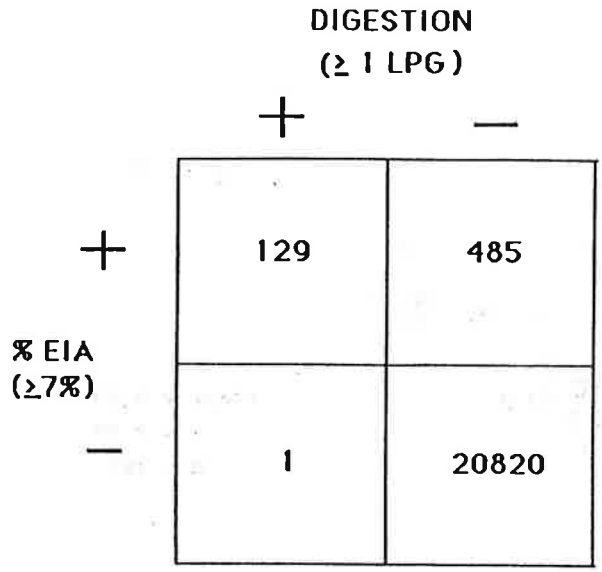

FIG. 2 EIA and tissue digestion analysis for hogs, 180 lbs. or larger

The lower limits of detection of sulfamethazine was 0.01 μg/mL. The accuracy of the method was checked by recovery experiments with sulfamethazine spiked samples. Recovery of sulfamethazine ranged from 97.6% to 112.5% (mean 103.3%) (Table 1).

Plasma and serum samples from 180 animals were evaluated by EIA. Values of sulfamethazine in these samples ranged from 0-6 ppm. The drug levels in plasma and serum from individual animals were similar with a high correlation of 0.98 and a slope of 0.93 (Fig.4).

Validity of our sulfamethazine test in plasma was checked by comparing with a standard chromatographic method (Williams 1984). Twenty-three field samples of plasma were quantitated by both EIA and TLC. Over 90% correlation was found between these two methods and the EIA results were comparable to those by the TLC (EIA = 0.93 TLC + 0.4). Day-to-day and within-day variability of the assay was less than 10%. The assay was very specific to sulfamethazine as among 30 other drug analogues tested only sulfamerazine showed significant cross-reaction (approx. 12%).

DISCUSSION

The EIA system allows an ideal tool for the management of trichina and chemical residues in a slaughterhouse. The system provides extremely sensitive, non-invasive and easily automated blood tests which are fast enough to keep up with kill speeds of thousands of animals per hour. Accuracy and precision of trichina and sulfamethazine EIAs are excellent. Both tests can be performed by using the same blood sample except that plasma or serum is required for sulfamethazine assay. Either serum or plasma of the animals can be used for quantitation of sulfamethazine. Correlation of sulfamethazine levels in hog tissues and serum have been established (Randecker et al. 1987) permitting the use of

readily available serum as a screening medium. Animal blood is easier than tissues to collect in a slaughterhouse, making this testing system more practical to use. Under development are tests for chloramphenicol, neomycin, penicillin, tetracycline, and pesticides.

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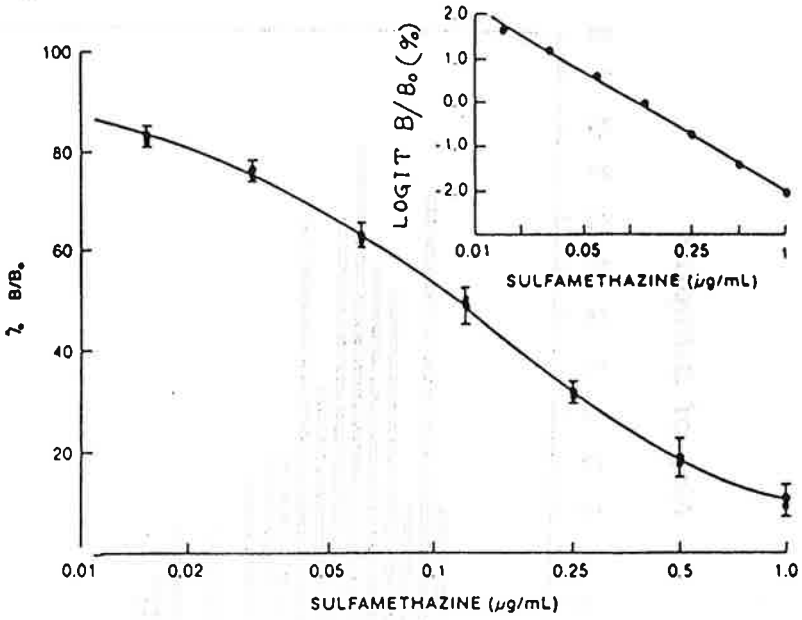

FIG. 3 Sulfamethazine EIA standard curve

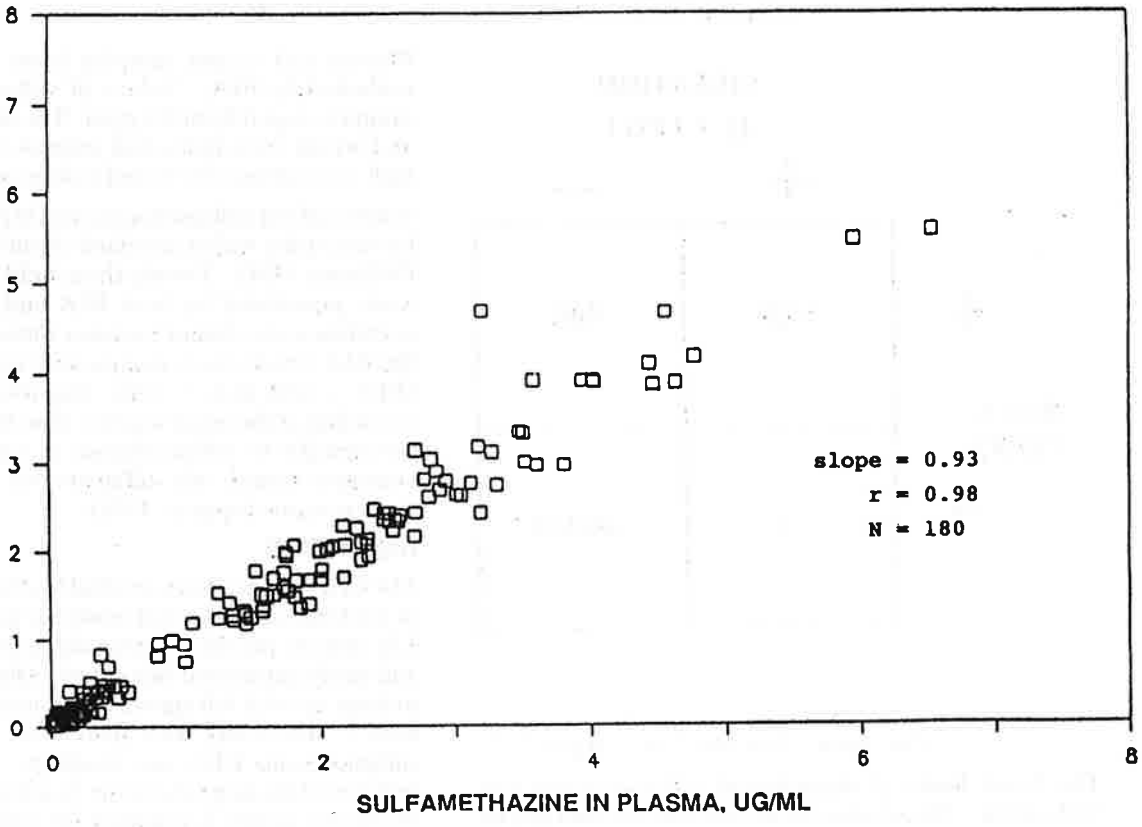

FIG. 4 Hog plasma vs. serum sulfamethazine by EIA. Samples provided by Dr. J. McKean, Iowa State University.