Comparison of Pepsin-digestion and Enzyme-linked Immunosorbent Assay for the Diagnosis of Trichinosis in Swine

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ABSTRACT

Comparison of parasitological and serological diagnosis of trichinosis in swine was carried out on 36 pigs given 15,400 infective larvae each by gavage. Circulating eosinophil levels were determined and sera were examined by enzyme-linked immunosorbent assay for anti-*Trichinella* antibodies. Two pigs were killed per day from days 15 to 29 postinfection. Muscle was examined by pepsin-digestion and comparable tissue was fed to a rat. Eosinophil counts increased at about day 6 and reached peak levels about day 25 postinfection and returned to approximate preinfection levels about two months postinfection in those pigs still in the study. Infective larvae were recovered from all pigs killed at ≥ 18 days postinfection. Using the criterion of 5 x mean optical density readings of negative sera as positive, seroconversion occurred between days 19 and 26 postinfection. Use of a lower criterion of 3 x mean optical density readings of negative sera resulted in only three of 30 pigs killed ≥ 18 days postinfection seroconverting ≤ 18 days postinfection, when infective larvae were first recovered in the musculature. In pigs, even in those heavily infected, there is a lag between the period that trichinae in musculature become infective and development of antibodies as detected by enzyme-linked immunosorbent assay which results in false negative reactions in many animals. This study demonstrated that the enzyme-linked immunosorbent assay using an excretory-secretory antigen should not be used to certify pork or pork products free of infective *Trichinella* larvae or safe for human consumption.

RÉSUMÉ

Cette expérience consistait à comparer le diagnostic parasitologique et sérologique de la trichinose, chez 36 porcs individuellement gavés avec 15,400 larves infectantes. L'auteur détermina le nombre d'éosinophiles en circulation et rechercha les anticorps sériques contre *Trichinella*, à l'aide de l'épreuve immunoenzymatique ELISA. Au bout de 15 à 29 jours après le gavage, il sacrifia quotidiennement deux porcs. Il examina leurs muscles par la digestion dans de la pepsine et en donna à manger à des rats. Le nombre d'éosinophiles augmenta, au bout d'environ six jours, et atteignit un sommet, au bout d'environ 25 jours; il retourna cependant au nombre approximatif d'avant l'infection, au bout d'environ deux mois, chez les porcs expérimentaux encore vivants. L'auteur recouvra des larves infectantes, chez tous les porcs sacrifiés, à compter de 18 jours ou plus après l'infection. En considérant comme positifs les échantillons de sérum dont la densité optique était cinq fois plus grande que celle des échantillons témoins, il conclut à l'apparition d'anticorps, au bout de 19 à 26 jours après l'infection. En considérant comme positifs les échantillons de sérum dont la densité optique n'était que trois supérieure à celle des échantillons témoins, seulement trois des 30 porcs abattus au bout de 18 jours ou plus après le gavage affichèrent des anticorps au bout de 18 jours ou moins après le gavage, alors que les muscles commençaient à arborer des larves. Chez les porcs, même chez les plus sévèrement infectés, il existe un laps de temps entre la période où les trichines musculaires deviennent infectantes et celle où l'ELISA permet d'en détecter les anticorps; il en résulte de fausses réactions négatives, chez plusieurs sujets. Cette expérience a démontré qu'on ne devrait pas utiliser l'épreuve ELISA dans laquelle on emploie un antigène composé d'excrétions et de sécrétions de trichines, pour certifier des carcasses ou des produits de porcs, exempts de larves infectantes de *Trichinella* ou propres à la consommation humaine.

INTRODUCTION

Studies carried out in 1985 showed the development of *Trichinella* antibodies in swine as directed by enzyme-linked immunosorbent assay (ELISA) to be dependent on the magnitude of the infection established, duration of the infection when animals were tested and the immunocompetence or response to infection of individual animals (1). Three pigs that had received 10,000 infective *Trichinella spiralis spiralis* larvae seroconverted between 23 and 30 days postinfection when tested by ELISA using an excretory-secretory (ES) antigen and a criterion of ≥ 5 x mean optical density (OD) readings of three negative swine sera as positive (1). The age when muscle larvae first become infective for a new host is variously stated to be between 17 and 21 days after infection (2). Considering the apparent delay

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Submitted March 2, 1987

between the time larvae in musculature become infective and antibody levels are sufficiently high to give a positive ELISA reading, a study was undertaken to investigate the lag between the infectivity of larvae in muscle and the development of antibodies in early heavy *T. spiralis spiralis* infections in swine.

MATERIALS AND METHODS

ANIMALS

York x Landrace pigs weighing approximately 20 kg were purchased from a commercial swine producer. Wistar laboratory rats were purchased from a commercial animal breeder. Pigs and rats were maintained on commercially prepared feed. The Canadian Council on Animal Care guidelines outlined in the "Guide to the Care and Use of Experimental Animals, Volume I" were followed.

EXPERIMENTAL DESIGN

Thirty-six pigs were each given an estimated 15,400 infective *T. spiralis spiralis* larvae via gavage. The *T. spiralis spiralis* was originally isolated from a pig in Nova Scotia and had been maintained in Wistar rats since 1974. Starting on day 15, two pigs were killed each day until day 29 postinfection. Two of the remaining six pigs were killed on day 33, two on day 46, one on day 77 and one on day 89 postinfection. From 417 to 865 g of musculature from each pig, including tongue, masseter, diaphragm, intercostal, psoas and rectus abdominis muscles were examined by the pepsin digestion procedure for the presence of infective *Trichinella* larvae.

Five grams of diaphragm from each pig killed between days 15 and 33 postinfection were fed to a rat to determine infectivity of the larvae. The rats were killed 30 to 35 days after being fed porcine diaphragm. Each rat was skinned, eviscerated and the whole carcass put through a meat grinder, digested and the number of larvae/rat determined.

Blood was collected in EDTA vacutainers (Becton Dickinson Canada, Mississauga, Ontario) for eosinophil determinations from pigs in the study on days 0, 1, 4-13 inclusive, 15, 18, 21, 25, 28, 33, 39, 43, 46, 50, 53, 56, 63, 77 and 89 postinfection.

Sera were examined for the presence of anti-*Trichinella* antibodies from pigs in the study on days 0, 7, 14-29 inclusive, 33, 39, 43, 46, 50, 53, 56, 63, 77 and 89 postinfection.

LABORATORY PROCEDURES

Pepsin-digestion of muscle was carried out in a 1% pepsin-1% HCl digestion mixture as previously outlined (1).

Eosinophil counts were carried out using Unopette disposable blood diluting pipettes (Becton Dickinson Canada, Mississauga, Ontario) and a hemacytometer.

Examination of sera for the presence of anti-*Trichinella* antibody was carried out using the ELISA triple antibody procedure of Gamble *et al* (3) but modified as previously described (1).

An OD reading at 414 nm $\geq$ 5 x the mean of three negative porcine sera was considered to be positive. Samples were also evaluated using criteria of 3 x and 4 x OD reading of the mean of three negative sera. Each serum was tested in duplicate.

RESULTS

PARASITOLOGICAL FINDINGS

Larvae were recovered from the musculature of all pigs examined by the pepsin-digestion procedure $\geq$ 18 days postinfection. The number of larvae recovered increased until approximately day 25 postinfection. In pigs killed $\geq$ 25 days postinfection, the mean infection established ranged from 131 to 580 larvae/g of musculature. In most pigs, the greatest number of larvae per gram of muscle was recovered from the diaphragm followed by the tongue and masseter. Fewer larvae were found in the intercostal, psoas and rectus abdominis muscles. Larvae were not found in the six pigs examined on days 15, 16 and 17 postinfection.

RAT BIOASSAY

The number of *T. spiralis spiralis* larvae recovered from rats fed 5 g of diaphragm of pigs infected with 15,400 larvae and killed from days 18 to 33 postinfection is given in Table I. Larvae were not recovered from rats fed 5 g samples of diaphragm from

TABLE I. *Trichinella* Larvae Recovered From Rats Fed 5 g Diaphragm of Pigs Given 15,400 Larvae and Killed From Days 18 to 33 Postinfection (PI)

Pig No.	Days PI Killed	Mean Larvae/g Diaphragm	Total No. of Larvae Recovered from Rat
7	18	9.5	1,392
8	18	1.0	981
9	19	71.4	1,386
10	19	8.8	510
11	20	157.4	47,200
12	20	268.5	7,380
13	21	312.2	16,800
14	21	97.2	34,100
15	22	200.6	93,600
16	22	53.1	115,700
17	23	96.1	14,800
18	23	110.3	19.700
19	24	405.1	139.500
20	24	274.5	164,000
21	25	556.2	165.500
22	25	587.3	36,500
23	26	928.0	196.500
24	26	929.2	216,000
25	27	564.5	41,208
26	27	824.0	44,200
27	28	499.5	52,300
28	28	851.0	72,400
29	29	1,443.9	140,904
30	29	420.0	63,024
31	33	384.7	116,857
32	33	678.4	136,900

pigs no. 1 to 6 that were killed on days 15, 16 and 17 postinfection.

EOSINOPHIL DETERMINATIONS

The mean eosinophil counts in pigs infected with 15,400 infective *T. spiralis spiralis* larvae and examined from days 0 to 89 postinfection are given in Table II. Eosinophil counts started to increase about day 6 and reached peak levels about day 25 postinfection. Eosinophil counts returned to approximately preinfection levels about two months postinfection.

SEROLOGICAL FINDINGS

Results of examinations of sera by ELISA from the 36 pigs are given in Table III. Using the criterion of 5 x mean OD readings at 414 nm of three negative sera as positive, 14 pigs had not seroconverted when killed between 15 and 21 days postinfection. The remaining 22 pigs serconverted between 19 and 26 days with a mean of 21.4 days postinfection. Using criteria of 3 x and 4 x mean OD reading of three negative sera, means for seroconversion were 19.9 and 20.8 days postinfection respectively. Once a sample reacted positively, the ELISA remained positive on subsequent serum samples from that animal.

TABLE II. Mean Eosinophil Counts in Pigs Infected with 15,400 Infective *T. spiralis spiralis* Larvae and Examined from Days 0 to 89 Postinfection (PI)

No. Pigs Examined	Days PI Examined	Eosinophils/μL Mean	Eosinophils/μL S.D.[a]
36	0	210	103.7
36	1	200	92.5
36	4	155	60.6
36	5	187	97.7
36	6	335	261.7
36	7	877	534.9
36	8	1048	715.0
36	9	969	662.3
36	10	1016	609.5
36	11	1423	898.0
36	12	1826	1378.0
36	13	2435	1612.9
36	15	3381	2077.7
30	18	2541	1777.4
24	21	2829	1473.9
16	25	4167	2937.7
10	28	3656	2764.5
6	33	3270	1827.3
4	39	1935	1156.4
4	43	1378	1012.3
4	46	1050	528.6
4	50	673	191.6
4	53	792	246.5
2	56	660	62.2
2	63	386	222.7
1	77	35	—
1	89	440	—

[a]Standard Deviation

DISCUSSION

In this study viable infective *T. spiralis spiralis* larvae were recovered from the musculature of all 36 pigs given a dose to 15,400 infective larvae and examined on days ≥ 18 postinfection. The infectivity of the larvae was confirmed by the rat-feeding trials which showed that transmission first occurred in rats fed muscle of pigs killed on day 18 postinfection. On the other hand, using the usual criterion of 5 x mean OD readings of three negative sera as positive, seroconversion in various pigs occurred from 19 to 26 days postinfection. Even when the criterion was lowered to 4 x and 3 x mean OD readings of negative sera, there was a lag between the time when muscle tissue first became infective and antibodies were detected by ELISA in most of the pigs. Using the low criterion of 3 x mean OD reading of negative sera as positive, only three of 30 pigs killed ≥ 18 days postinfection had seroconverted by day 18 postinfection, when trichinae were first shown to be infective. This study confirms previous observations that there may be a period early in the infection when the muscle is infective but false negative ELISA reactions are recorded (1,4).

This trial also demonstrates the variable immune response of individual animals to infection. For example, pig 29 which did not seroconvert at 5 x mean OD readings of negative sera until day 26 postinfection, had a mean infection of 580.6 larvae/g of muscle (the heaviest infection established in any of the pigs).

The circulatory eosinophilia established in the pigs started to develop about six days postinfection and reached a peak at about day 25 postinfection which coincided with the period when muscle invasion had reached maximal levels based on mean number of trichinae in muscle of pigs examined in this study. Approximately two months postinfection, the eosinophilia had gradually returned to preinfection levels in those pigs still in the trial in spite of the fact that heavy muscle infections were still present. These results suggest that a circulatory eosinophilia is associated with invasion of larvae into the musculature. It would seem that in the pig, at least, eosinophil determinations may be a reliable aid to diagnosis only during the early phases of the infection up to about six weeks postinfection.

TABLE III. Serological Findings in Pigs Given 15,400 *T. spiralis spiralis* Larvae and Examined by ELISA Using an ES Antigen and Positive Criteria of 3x, 4x or 5x OD Mean Readings at 414 nm of Three Negative Sera

Days Postinfection (PI) Pigs Killed	First Positive ELISA Reading (Day PI) 3 x OD	4 x OD	5 x OD
15	-/-[a]	-/-	-/-
16	-/-	-/-	-/-
17	17/-	-/-	-/-
18	-/-	-/-	-/-
19	19/-	-/-	-/-
20	20/20	20/20	-/-
21	-/21	-/-	-/-
22	19/20	19/21	20/21
23	19/21	21/21	21/23
24	18/19	19/21	19/22
25	14/21	20/21	20/22
26	20/20	20/21	21/21
27	19/20	20/21	20/21
28	21/22	21/22	22/22
29	22/25	22/25	22/26
33	20/20	21/21	21/21
46	20/20	20/20	21/21
77	18	21	21
89	22	22	23

[a]Where two pigs were tested, results on each are given as -/-, etc; - indicates that all sera were negative to the time the pig was killed

A predilection site for *T. spiralis spiralis* in most of the pigs in this study was the diaphragm followed by the tongue and masseter muscles. As in the horse (4), the tongue muscle would appear to be a reliable organ to examine for the presence of *Trichinella* larvae.

In summary, this study demonstrates that the ELISA cannot be used to certify pork products free of *T. spiralis spiralis*. Even in heavy infections there may be a lag of several days between the time the larvae in the musculature are infective and antibodies are detected by ELISA.

ACKNOWLEDGMENTS

The author is indebted to Mr. K.E. Snowdon for his technical assistance. The assistance of Messrs D.R. Green, R.D. Beal and M.J. Shea in the collection of specimens is much appreciated.

REFERENCES

1. **SMITH HJ.** Evaluation of the ELISA for the serological diagnosis of trichinosis in Canadian swine. Can J Vet Res 1987; 51: 194-197.
2. **VILLELLA JA.** Life cycle and morphology. In: Gould SE, ed. Trichinosis in Man and Animals. Springfield, Illinois: Charles C. Thomas, Publisher, 1960: 19-60.
3. **GAMBLE HR, ANDERSON WR, GRAHAM CE, MURELL KD.** Diagnosis of swine trichinosis by enzyme-linked immunosorbent assay (ELISA) using an excretory-secretory antigen. Vet Parasitol 1983; 13: 349-361.
4. **SMITH HJ, SNOWDON KE.** Experimental *Trichinella* infections in ponies. Can J Vet Res 1987; 51: 415-416.