

## THE LATERAL FLOW CARD TEST: AN ALTERNATIVE METHOD FOR THE DETECTION OF *TRICHINELLA* INFECTION IN SWINE

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### Summary :

A novel lateral flow card (TS-Card pork) test was developed for the serological detection of *Trichinella* infected pigs. Based on extensive studies performed in Romania during 1999-2000 this test proved to be highly specific, sensitive, rapid (3-12 minutes) and easy to use (no need for laboratory facilities). It can be used both for the detection of *Trichinella* infection in carcasses and for epizootiological studies using a variety of samples including whole or dried blood, serum, or tissue fluids. The TS-Card pork test, used as a screening test, can be the foundation of an on-farm or field based inspection system to significantly improve food safety in countries with a high prevalence of *Trichinella* in pigs or other food animal species. The results presented are also promising for application of the test in an on-line laboratory based inspection system since the speed of the test allows sufficient time to rail out suspected hog carcasses during the slaughter process.

**KEY WORDS :** serology, *Trichinella*.

IVD Research Laboratories, USA and A.R.T.E. SRL, Romania developed a novel, simple and rapid TS-Card pork lateral flow test for detection of *Trichinella* infection in swine. A field evaluation of the TS-Card pork test was performed in an extensive, double blind study in Romania. The focus of the study was comparison of two diagnostic methods, the TS-Card pork (a serological method) and trichinosis (a parasitological method), in their efficiency for the routine control of trichinellosis in pigs. The presence of *Trichinella* infection in the pork was verified using the artificial digestion method. The results are presented in this communication.

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## MATERIALS AND METHODS

### SWINE

A total of 1,098 animals from the Timis, Prahova and Teleorman districts of Romania were included in this study. Of this group, 635 pigs originated from *Trichinella* non-endemic farms (Timis and Teleorman districts) and 463 pigs originated from *Trichinella* endemic farms (Prahova district; prevalence of *Trichinella* infection in pigs reported to be over 20 % in the last three years).

### BLOOD AND SERUM SAMPLES

Whole blood and serum samples were collected from all pigs examined in the study. Blood samples were collected in EDTA coated tubes while blood for serum was collected in non-coated tubes. All samples were labeled according to the protocol.

### MEAT SAMPLES

A sample of diaphragm pillar (crus) tissue was collected for trichinosis and for pooled or individual peptic digestion testing.

### PROTOCOL FOR THE DOUBLE BLIND STUDY

The blood, serum and meat samples used for the double blind studies were identified by tag number and recording number. Samples were tested by recording number and were identified by tag number after trichinosis, TS Card pork test, and peptic digestion assay. The Romanian Ministry of Agriculture conducted this project through the National Sanitary Veterinary Agency and the Institute for Diagnosis and Animal Health, Bucharest, Romania. A Romanian official, representing the independent State Institutions signed the final report as proof that the study was performed as a double blind.

### TRICHINOSCOPY METHOD

Trichinosis was done according to European Economic Community Commission Directive 84/319/EEC

Animals examined from	TS-Card pork positive/tested (%)	Trichinoscopy positive/tested (%)	Peptic digestion positive/tested (%)
<i>Trichinella</i> non-endemic farms	3/635 ( 0.47)	0/635 (0)	0/635 (0)
<i>Trichinella</i> endemic farms	93/463 (20.09)	60/463 (12.96)	93/463 (20.09)
Total	96/1098 (8.74)	60/1098 (5.45)	93/1098 (8.47)

Table I. – Comparative efficacy of serological (TS-Card pork) and direct parasitological methods for the detection of *Trichinella* infection in pigs in Romania.

and the OIE Manual of Standards for Diagnostic Tests and Vaccines (2000). The 28 fields of one compressorium were examined for each sample.

#### PEPTIC DIGESTION METHOD

This method was performed as described in OIE Manual of Standards for Diagnostic Tests and Vaccines (2000), Chapter 3.5.3.

#### IDENTIFICATION OF THE *TRICHINELLA* GENOTYPE

The Istituto Superiore di Sanita, Rome, Italy, conducted the genotype analyses of infective *Trichinella* muscle larvae as previously described (Pozio *et al.*, 1997).

#### TS-CARD PORK LATERAL FLOW TEST

This test was performed according to the manufacturer's instructions (IVD Research Laboratories, USA and A.R.T.E. SRL, Romania).

#### ANTIBODY DETECTION

ELISA (Gamble, 1998) and Western blot (Laemmli, 1970; Towbin *et al.*, 1979) analyses, using ES antigen and anti-swine-HRP, were performed simultaneously at INEP (Belgrade, Yu) and ISS (Rome, Italy).

## RESULTS

The results obtained for the comparative analyses of the efficacy of the serological (TS-Card pork) and direct parasitological methods for detection of *Trichinella* infections in pigs in Romania are presented in Table I.

If two bands were visible, the sample was positive; if there was only one band (control band) the test was negative. The test took from three minutes (for the appearance of a specific band with very strong positive sera) up to 12 minutes (for very weak positive sera).

Using the TS-Card pork test, 632 sera were identified as negative and three sera (0.47 %) as positive. Using two other serological tests, ELISA and Western blot,

Results of tests performed for determination of <i>Trichinella</i> infection in pigs	No.	%
Positive Trichinoscopy/Positive TS-Card	60/93	64.52
Negative Trichinoscopy/Positive TS-Card	33/93	35.48
Positive Trichinoscopy/Negative TS-Card	0	0
Negative Trichinoscopy/Negative TS-Card	0	0

Table II. – Comparative analyses of results obtained by TS-Card pork and trichinoscopy in pigs that were positive by peptic digestion.

specific antibodies against *Trichinella* were not seen in any of the 635 samples. All 635 animals were negative by trichinoscopy and by peptic digestion (as a confirmatory assay). Based on these results, the three pigs testing positive by TS-Card pork were considered to be false positives. Results obtained with the TS-Card pork test on 463 pigs from *Trichinella* endemic farms revealed that 93 (20.09 %) animals were seropositive. A significant discrepancy was seen in positive results obtained by trichinoscopy (12.96 %) and peptic digestion (20.09 %). Using the TS-Card pork test, there were no false positive or false negative results on swine originating from *Trichinella* endemic farms when compared with results of peptic digestion.

Results comparing the efficiency of the TS-Card pork test and trichinoscopy for the routine control of trichinellosis are presented in Table II.

Trichinoscopy identified only 60 (64.52 %) of the 93 pigs that were positive by peptic digestion. At the same time, the TS-Card pork test identified all infected animals. Peptic digestion analyses pointed out that in *Trichinella* endemic regions in Romania the infectivity level was variable with worm burdens ranging from 0.1 LPG to 44 LPG. Of the larvae recovered from pigs, it was determined that most isolates were whether *T. spiralis* and *T. britovi*.

Additional results obtained by TS-Card pork on all analyzed animals showed that there were no discrepancies in two or more analyses of one serum or blood sample originating from the same pig, as well as between results obtained using either serum or blood from the same pig (100 % compatibility on sera and blood results by TS-Card pork; 100 % test reproducibility).

## DISCUSSION

In this study it was established that even very low level infections with *Trichinella* (0.1 LPG of muscle), can be detected using the TS-Card pork test. Study of the applicability of the TS-Card pork test for the detection of pig infections in *Trichinella* endemic regions, points out that the test is very specific because of the absence of either false negative or false positive results. The results obtained demonstrating a very low rate of false positive, results in swine from non-endemic regions of Romania should be examined further. Additional research will be need to establish methods for evaluating weak and strong bands on TS cards. In fact, preliminary results suggest that the appearance of bands with different intensity is confusing for the reader (Pozio E., unpublished data). Furthermore, the sensitivity of the TS-card test in the window period (i.e., from 15 and 21 days after infection when larvae of *T. spiralis* can infect a new host, but serological tests could be negative) should be evaluated. The reproducibility of the TS-Card pork test is very high.

The TS-Card pork test is very easy to perform. No equipment is needed and it can be performed in the field, in small veterinary clinics or virtually anywhere else. The results obtained can be saved for confirmation.

Based on the favorable results obtained in this study, the TS-Card pork test has a variety of potential uses. It can be used for identifying infected animals and dealing with them on an individual basis or it can be used for epizootiological studies. It is a tool that could be used by slaughterhouses to test animals that are suspected to originate from farms with endemic trichinellosis, with the aim of reducing expenses of buying, slaughtering and discharging meat of infected animals or as a method to rapidly identify individually infected animals when positive results are obtained by pooled digestion (thus reducing time and expense due to the long procedure of digestion re-examination).

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