Investigation on *Trichinella* spp. in Swine in Eastern Bolivia

FABIO MACCHIONI[1]
MARTA MAGI[2]
LISA GUARDONE[3]
FRANCESCO TOLARI[4]
FABRIZIO BRUSCHI[5]
SIMONA GABRIELLI[6]
RAÚL LÓPEZ RAMOS[7]
REINALDO GUZMÁN RÍOS[8]
LIMBERG ROJAS GUZMÁN JOS[9]
LUIS QUIROGA CIVERA[10]

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Abstract

In Bolivia trichinellosis in swine was documented for the first time in 1993 in a small abattoir of a rural community in the Bolivian Altiplano, with the pooled digestion method. In 1996 a second survey was performed using serological tests to detect antibodies against *Trichinella spiralis* in pigs in the departments of Santa Cruz and Chiuquisaca. No further investigations have been performed since then. Between July and September 2011, a total of 65 swine muscular samples, 50 from a slaughter house in Santa Cruz and 15 from the slaughter house of Camiri (eastern Bolivia) were examined to detect *Trichinella* using the artificial digestion method**.** Furthermore a serological survey on a total of 255 serum, collected in 2007 from the community of Bartolo (Hernando Siles province, Department of Chuquisaca), Monteagudo, and Chuquisaca (Department of Chuquisaca) was performed using two commercial indirect ELISA kits that use excretory/secretory antigens (ESA) of *Trichinella* spp. Six out 255 resulted positive with an overall seroprevalence of 2.3%. None of the 65 pig muscular samples analyzed with artificial digestion tested positive for the presence of *Trichinella* larvae. These investigations indicate that trichinellosis is present throughout Bolivia and is a potentially important public health problem.

1 Biologist, Department of Animal Pathology Prophylaxis and Food Hygiene. Faculty of Veterinary Medicine of Pisa, Viale delle Piagge 2, 56127 Pisa, Italy. E-mail: fmacchion@vet.unipi.it

2 MV, Department of Animal Pathology Prophylaxis and Food Hygiene. Faculty of Veterinary Medicine of Pisa, Viale delle Piagge 2, 56127 Pisa, Italy. E-mail: magim@vet.unipi.it

3 MV, Department of Animal Pathology Prophylaxis and Food Hygiene. Faculty of

Veterinary Medicine of Pisa, Viale delle Piagge 2, 56127 Pisa, Italy. E-mail: lisa.guardone@for.unipi.it

4 MV, PhD, Department of Animal Pathology Prophylaxis and Food Hygiene. Faculty of Veterinary Medicine of Pisa, Viale delle Piagge 2, 56127 Pisa, Italy. E-mail: tolari@vet.unipi.it

5 Physician, PhD, Department of Experimental Pathology, Medical Biotechnologies, Infectious Diseases and Epidemiology. Faculty 'of Medicine and Surgery Via Roma 55, 56126 Pisa, Italy. E-mail: fabrizio.bruschi@med.unipi.it

6 Biologist, PhD, Department of Public Health Sciences "G. Sanarelli ". Faculty 'of Medicine and Surgery, Piazzale Aldo Moro 2, 00185 Roma, Italy E-mail: simona.gabrielli@uniroma1.it

7 MV, Responsable de Parasitologia, Laboratorio Lidivet, Avenida Ejército Nacional # 153 Santa Cruz de la Sierra, Bolivia. E-mail: raullr20@hotmail.com

8 MV, Responsable de Bioquimica, Laboratorio Lidivet, Avenida Ejército Nacional # 153 Santa Cruz de la Sierra, Bolivia. E-mail: guzman_reinaldo@hotmail.com

Keywords

Trichinellosis, Swines, Bolivia, ELISA Test, Artificial Digestion

INVESTIGACIÓN SOBRE LA *TRICHINELLA* SPP. EN LOS CERDOS EN EL ESTE DE BOLIVIA

Resumen

En Bolivia, la triquinosis en los cerdos se documentó por primera vez en 1993, tras un estudio en un pequeño matadero de una comunidad rural utilizando el método de digestión artificial, y tras una investigación en 1996 mediante pruebas serológicas para detectar la presencia de anticuerpos contra *Trichinella spiralis* en cerdos en los departamentos de Santa Cruz y Chiuquisaca. No hay más registro de otras investigaciones. Entre julio y septiembre de 2011, un total de 65 muestras se analizaron con el método de digestión artificial para detectar las larvas de *Trichinella* sp., de las cuales ninguna muestra resultó positiva. Además, se realizó un estudio serológico en un total de 255 sueros recogidos en 2007 de la comunidad de Bartolo (provincia Hernando Siles del departamento de Chuquisaca), Monteagudo y Chuquisaca (departamento de Chuquisaca) mediante dos kits comerciales de Elisa indirecto que utilizan excretor/secretor/antígenos (ESA) de triquinosis; 6 de las 255 muestras resultaron positivas, con una seroprevalencia de 2,3%. Ninguna de las 65 muestras musculares de cerdo analizadas con la digestión artificial resultó positiva para la presencia de larvas de *Trichinella*. Estas investigaciones indican que la triquinosis está presente en Bolivia y es un problema de salud pública potencialmente importante.

Palabras clave

Triquinosis, cerdos, Bolivia, prueba de Elisa, digestión artificial.

PESQUISA SOBRE A *TRICHINELLA* SPP. EM PORCOS NO LESTE DA BOLÍVIA

Resumo

Na Bolívia, a triquinose nos porcos foi documentada pela primeira vez em 1993, depois de um estudo em um pequeno matadouro de uma comunidade rural utilizando o método de digestão artificial, e depois de uma pesquisa em 1996 mediante testes sorológicos para detectar a presença de anticorpos contra *Trichinella spiralis* em porcos nos departamentos de Santa Cruz e Chiuquisaca. Não há mais registro de outras pesquisas. Entre julho e setembro de 2011, um total de 65 amostras foram analisadas com o método de digestão artificial para detectar as larvas de *Trichinella* sp., das quais

nenhuma amostra deu positivo. Além disso, realizou-se estudo sorológico em um total de 255 soros recolhidos em 2007 da comunidade de Bartolo (província Hernando Siles do departamento de Chuquisaca), Monteagudo e Chuquisaca (departamento de Chuquisaca) mediante dois kits comerciais de Elisa indireto que utilizam excretor/secretor/antígenos (ESA) de triquinose; 6 das 255 amostras deram positivo, com uma soroprevalência de 2,3%. Nenhuma das 65 amostras musculares de porco analisadas com a digestão artificial deu positivo para a presença de larvas de *Trichinella*. Estas pesquisas indicam que a triquinose está presente na Bolívia e é um problema de saúde pública potencialmente importante.

Palavras chave

Triquinose, porcos, Bolívia, teste Elisa, digestão artificial.

9 MV, Responsable de Virología y Biología Molecular, Laboratorio Lidivet, Avenida Ejército Nacional # 153, Santa Cruz de la Sierra, Bolivia. E-mail: limberg_rg3@yahoo.com

10 MV, Responsable de Enfermedades Vesiculares y del Cerdo, Lidivet Avenida Ejército Nacional # 153, Santa Cruz de la Sierra, Bolivia. E-mail: jlqc2003@hotmail.com

Introduction

Trichinellosis is a zoonosis diffused all over the world, with the exception of the Antarctic continent. The *Trichinella* infection has been reported in South and Central America (México, Argentina, Chile) in pigs, horses, rats, cats and dogs and in humans since the end of the 19th century.

In Argentina 5221 human cases were reported (average incidence of 1.5) between 1990 and 2005, all caused by domestic pork consumption (Murrell & Pozio, 2011). In Chile between 1991 and 2004, 698 human cases were reported (average incidence of 0.4). In every case the source of the infection was identified as pork consumption (Murrell & Pozio, 2011). In both countries *Trichinella spiralis* has been found in domestic and wild animals (Ortega Pierres et al., 2000). In Uruguay and in Venezuela, there are reports of trichinellosis in cadavers as well as living individuals (Neghme & Schenone, 1970; Acha & Szyfres, 1986). Information remains unavailable from Colombia, Costa Rica, Guatemala, Ecuador, Paraguay and Perú (Pozio, 2007).

In Bolivia, trichinellosis in pigs was documented for the first time by Bjorland et al. (1993) with the pooled digestion method. A second study in 1996 was conducted on swine serum (Brown et al., 1996) and in 1999 a serological screening of humans in Bolivia conducted by Bartoloni et al. (1999) revealed the

presence of antibodies against *Trichinella*. There is no information is available on the species of *Trichinella* circulating in Bolivia. The aim of this investigation is to perform a preliminary serological screening on pig sera using two different commercially available ELISA kits and to possibly identify *Trichinella* larvae in pooled muscular pig samples.

Materials and Methods

Artificial Digestion

Between July and September 2011, a total of 65 swine muscular samples, 50 from a slaughter house in Santa Cruz and 15 from the slaughter house of Camiri (Eastern Bolivia) were examined using the artificial digestion method to detect *Trichinella* larvae according to the European Commission Regulation (EC) N. 2075/2005 protocol (Thomsen, 1978, 1997). The digestion was carried out for 25 minutes at 41°C using pepsin 1:10.000 U.I (SIGMA-Aldrich) and 17.5% HCl followed by filtration with a sieve and microscopic examination. The tissue samples were collected from the pilaster of the diaphragm of slaughtered pigs and were pooled as follows: five pools of 10 samples with 10 grams for each of the samples from Santa Cruz; and three pools of five samples with 20 grams for each of the samples collected in Camiri (Department of Santa Cruz).

Serology

Serology on swine sera was performed using two commercial indirect ELISA kits that use excretory/secretory antigens (ESA) of *Trichinella*: PIGTYPE® *Trichinella* Ab (Labor Diagnostik, Leipzig) and ID Screen® *Trichinella* Indirect Multi-species (IDvet innovative diagnostics, Montpellier - FRANCE). Tests were carried out following the manufacturer's instructions. The first group's sample breakdowns are as follows: 87 sera were examined using PIGTYPE® *Trichinella* Ab: 51 of those serum samples were collected in 2007 from living pigs in the community of Bartolo (Hernando Siles province, Department of Chuquisaca); 20 sera were collected at the slaughterhouse of Monteagudo, and 16 were collected at the slaughterhouse of Chuquisaca (Department of Chuquisaca). A second group of 168 sera (40 collected at the slaughterhouse of of Monteagudo and 128 at the slaughterhouse of Chuquisaca) were analyzed with the ID Screen® *Trichinella* Indirect Multi-species kit.

Results

None of the 65 muscular pig samples analyzed with the artificial digestion tested positive for the presence of *Trichinella* larvae. Concerning the serological investigation, six out of the 255 serum samples examined tested positive using the aforementioned Elisa kits (with an overall seroprevalence of 2.3 %). PIGTYPE® *Trichinella* Ab detected two positive samples among the 51 pig sera collected in Bartolo and one positive among the 20 sera from Monteagudo. ID Screen® *Trichinella* Indirect Multi-species detected three positive sera among the 128 sera collected in Chuquisaca.

Discussion

Trichinellosis in pigs is linked to the environment in which these animals are raised and the feed they receive. Pigs can become infected after eating meat scraps from domestic and wild pigs, and/or after eating the meat of infected rodents or carnivores. Most of the pigs in Bolivia are still raised in villages, fed with foraging and household waste and therefore they are increasingly susceptible to the *Trichinella* infection. Trichinellosis in pigs is generally sub-clinical and infected pigs can be slaughtered regularly. In Bolivia they are often slaughtered without any veterinary control and this can further increase risk for human infection.

In Bolivia trichinellosis in pigs was documented for the first time by Bjorland et al. (1993). In this study *Trichinella* was detected by the pooled digestion method (two out of eight pools were positive) and by serology, using an Elisa test (11.2 % seroprevalence). Serological research on pig sera collected in the abattoirs of Monteagudo, Vallegrande and Santa Cruz in the departments of Santa Cruz and Chuquisaca (Eastern Bolivia), detected a seroprevalence of 12.4 %, 17.1 % and 10.2 % respectively (Brown et al., 1996). An initial study on the presence of antibodies against *Trichinella* in humans in Bolivia performed on 234 individuals in the rural area of the Cordilera province in the Santa Cruz department, showed a seroprevalence of 3 % (Bartoloni et al., 1999). According to our serological screening a small proportion of pigs tested positive for antibodies against ESA of *Trichinella* spp. The majority of the positive samples were collected in villages and communities where pig farming is carried out in a traditional way, as in Monteagudo, Chuchisaqua and the community of Bartolo. Unfortunately, artificial

digestion could not be conducted on tissue samples from the serologically positive animals, representing the major limitation of our study. Muscular samples had not been collected at the time of the serological sampling because up to now artificial digestion is not a routinely performed exam. Nevertheless, the results of our serological investigation, together with the previous surveys, suggest that trichinellosis is present throughout Bolivia and may be a potentially important public health problem. Our attempts to detect and identify *Trichinella* larvae in pig muscular samples were concentrated in a slaughterhouse of Santa Cruz in which slaughtered pigs are raised in industrial farms. Only a small proportion of the examined muscle samples came from the area of Camiri, where pig production is more traditional. Therefore, it is not surprising that given our small sample size, all of the examined samples tested negative. It would have been interesting to perform a serological examination of the slaughtered pigs from which muscle samples were taken, but unfortunately this was not possible. We hope that this preliminary investigation will lead to future studies with larger sample sizes, particularly in those areas where positive samples were detected.

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