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Candidates for reference swine serum with anti-*Trichinella* antibodies

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ABSTRACT

Serology to monitor *Trichinella* spp. infection in pigs reared in controlled system has been claimed as a possible diagnostic tool. However, no international biological standards or reference materials exist to validate in house tests or commercial kits, and to improve the inter-laboratory comparability for the serological detection of anti-*Trichinella* IgG in pigs. In this work, potential reference sera have been prepared from four experimentally infected pigs. Sera were tested, aliquot, lyophilized, and maintained at +4 °C. Since one of the prerequisites for the development of any reference material is to plan and execute stability studies, isochronous studies for short and long term stability testing were carried out to evaluate the possible degradation effects of transportation and storage. The stability of the lyophilized serum samples at +4 °C, was arbitrarily assumed. For the short term stability study, two units were stored at −20 °C, +4 °C, +20 °C, and +50 °C for 0, 1, 2, and 4 weeks, and then tested in duplicate. For the long term stability study, the same number of units and replicates per unit were stored at −80 °C, −20 °C, and +4 °C for 0, 6, 12, 18 and 24 months. In both studies, unit samples were selected randomly and tested on the same day under repeatability conditions. The linear regression versus time for each serum at each studied temperature was analyzed and then slopes were tested for significance. Further, uncertainty of the short and long term stability was calculated for a shelf life period of one week and three years, respectively. For all sera but one, and for all the studied temperatures but +50 °C, the data from the short term stability study indicate the absence of a significant trend that would hint at degradation. The slopes of the regression lines did not significantly vary from zero. Even if the uncertainty of the short term stability was variable among serum samples, the rate of degradation was considered acceptable. For the long term stability, slopes of the regression lines of two serum samples significantly varied from zero, indicating a trend of possible degradation during storage. The percentage of degradation deducted from the uncertainty of the long term study varied; however, two serum samples showed the lower rate of degradation at all the assayed temperatures. The most suitable temperatures for dispatching serum samples are −20 °C, +4 °C and +20 °C; whereas, −20 °C and −80 °C are suitable temperatures for serum storage.

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1. Introduction

Serology to monitor *Trichinella* spp. infection in pigs reared in controlled systems has been claimed as a possible diagnostic tool (Gamble et al., 2004; World Organization for Animal Health, 2012). However, no international biological standards (IBS) or reference materials (RM) exist to validate in house tests and commercial kits, or to improve the inter-laboratory comparability for the serological detection of anti-*Trichinella* IgG in pigs (Gómez-Morales et al., 2009, 2012).

The World Health Organization (WHO) defines IBS and RM as substances of biological or synthetic origin that cannot be characterized adequately by chemical and/or physical means alone and that are used in the prophylaxis, treatment or diagnosis of human and animal diseases (WHO, 1986). According to the World Organization for Animal Health, the term International Reference Standard is synonymous with primary reference standard. It represents the standard by which all others standards are compared and calibrated (http://www.oie.int/en/our-scientific-expertise/veterinary-products/reference-reagents).

For parasite infections, no diagnostic test or RM have been standardized yet, except for a human serum of anti-*Toxoplasma* IgG (human TOXM, NIBSC, United Kingdom) (Rigsby et al., 2004). In the absence of gold standard methods, the availability of IBS and or RM could help in the validation and harmonization of serological methods for parasite infections. IBS such as serum samples are crucial for the validation of a serological test for detecting antibodies as an internal quality control and for the determination of accuracy and reproducibility. Moreover, their use should improve the inter-laboratory comparability of the results (Wright, 1998).

In the field of zoonotic foodborne parasites, only nematodes of the genus *Trichinella* are specifically investigated at the slaughterhouse by a direct test, namely the artificial digestion, which is the only internationally accepted gold standard to detect these parasites in susceptible animals, mainly pigs (World Organization for Animal Health, 2012). Serological methods, in particular ELISA, aimed to detect anti-*Trichinella* IgG in pigs, could be suitable for surveillance and epidemiological investigation of animal populations (Gamble et al., 2004; European Commission, 2005). In the last decade, it has been shown that *Trichinella* spp. do not circulate in pigs reared in controlled systems, and are confined to backyard or free-ranging pigs and to wild animals (Pozio, 2014). In controlled systems, serological tests could be used to monitor a percentage of pigs or when a pig population is moved from non-controlled to controlled farms. Unfortunately, no IBS or RM are available for this parasitic infection, thus the standardization of serological methods is difficult.

Even if twelve different taxa are recognized in the genus *Trichinella* (Pozio and Zarlenga, 2013), their antigenic pattern is quite similar, thus the antigens prepared from one taxon can be used to detect specific antibodies in hosts infected with different taxa (Gamble et al., 2004; Ortega-Pierres et al., 1996).

The aim of this work was to prepare a panel of swine sera with anti-*Trichinella* IgG as IBS or RM candidates, and to evaluate their stability by short and long term isochronous studies to estimate the possible degradation of the material during transportation and storage.

2. Materials and methods

2.1. Pig sera

Six pigs (*Sus scrofa domesticus*) were individually housed under biosafety conditions. After one week, four pigs were infected with 20,000 (p116) or 30,000 (p114, p115, and p119) *Trichinella spiralis* muscle larvae collected by artificial digestion from three-month-old infected mice. In order to monitor the sero-conversion and the presence of detectable anti-*Trichinella* IgG in serum samples by ELISA, blood samples were harvested from all pigs at time 0 and then daily after the 15th day post infection (p.i.) (Gómez-Morales et al., 2009). One infected pig (p119) was slaughtered the first day after sero-conversion, two pigs (p114 and p115) two days after sero-conversion, and the other three pigs (one infected, p116, and two non-infected control animals) were sacrificed at day 45 p.i. (Table 1). The number of muscle larvae per gram (LPG) was determined in 50–100 g samples of diaphragm, tongue and masseter by the magnetic stirrer method according to the Commission Regulation No. 2075/2005 (European Commission, 2005). Blood from the six animals was collected at slaughtering, allowed to clot and serum samples were obtained from each animal. After ELISA testing, samples were distributed in 0.5 mL aliquots, frozen at −80 °C, lyophilized at the FATRO plants (Ozzano Emilia, Bologna, Italy), sealed under vacuum, and stored in the dark. The number of serum sample aliquots was 930 and 4351 from uninfected (codes SCp+117 and SCp+118) and infected (codes SCp+114, SCp+115, SCp+116, and SCp+119) pigs, respectively. Storage at +4 °C was arbitrarily assumed to provide stability to serum samples, which, therefore, were kept at this temperature. Furthermore, it was arbitrarily estimated that lyophilized serum samples stored at +4 °C, have a shelf life of three years.

2.2. ELISA

Each serum sample was reconstituted in water of analytical grade, and tested in duplicate by a validated protocol (Gómez-Morales et al., 2009). Since raw optical density (OD) values are absolute measurements that are influenced by ambient temperature, test parameters, and photometric instruments, the results were expressed as a function of the reactivity of the positive control serum sample with the highest value among the four control sera included in each run of the assay. This control must yield a result that is in the linear range of measurement (World Organization for Animal Health, 2012). The mean OD values of the control sera, as well as the mean OD values of the duplicate test sera, were then calculated, and for each serum an ELISA