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Suitability of a *Salmonella* control programme based on serology in slaughter heavy pigs

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ABSTRACT

The key component of most European pig *Salmonella* control programmes is the classification of herds according to seroprevalence at slaughter. The objectives of this study were to estimate the true *Salmonella* seroprevalence, and investigate the association between the true status of infection and serology in slaughter heavy pigs. Blood of 3340 pigs was collected and tested with ELISA. From 385 pigs, also lymph nodes and cecal content were collected for bacteriology. Analysis was performed in a Bayesian framework.

Results showed that a large proportion of pigs was serologically positive (herd seroprevalence 93% and within-herd seroprevalence higher than 81% in half of herds at cut-off 10 OD%). The association between the true status of infection and serology was not significant, and therefore the classification of heavy pig herds according to seroprevalence at slaughter would not be suitable to reduce the risk of introducing *Salmonella* into the food chain.

1. Introduction

In the European Union (EU), *Salmonella* is the second most common agent of foodborne disease in humans (European Food Safety Authority, European Centre for Disease Prevention and Control, 2014). Eggs and poultry meat are recognized as the major sources of infection, mostly caused by *Salmonella enterica* subsp. *enterica* serovar *Enteritidis* (European Food Safety Authority, European Centre for Disease Prevention and Control, 2014). The incidence in humans has fallen by one third between 2008 and 2011, and this success has been attributed to the effectiveness of control measures in poultry (European Food Safety Authority, European Centre for Disease Prevention and Control, 2013). The consumption of pork products was also implicated in a number of *Salmonella* outbreaks mostly due, in this case, to *S. enterica* subsp. *enterica* serovar *Typhimurium* (European Food Safety Authority, European Centre for Disease Prevention and Control, 2013).

The EU Regulation No 2160/2003 requires Member States to reduce *Salmonella* prevalence in pork. Member States have to provide information on *Salmonella* prevalence in pigs and identify point of infection and factors which favor *Salmonella* presence at the farm in order to develop specific control strategies. Additionally, they should establish the bacterial status of herds entering the processing plants, and investigate the level of carcass contamination.

Many EU countries like Denmark, Sweden, Germany, Ireland, Belgium, and the Netherlands have been running a national *Salmonella* control programme focused on primary production (farm) as well as other intervention points (transport lairage, slaughter and processing) to reduce *Salmonella* prevalence in pigs and pork products (National Reference Laboratory, Department of Agriculture, Food and the Marine, Ireland, 2011).

Control programmes can be either voluntary or compulsory, governmental or not (European Food Safety Authority (Panel on Biological Hazards), 2006). The key element of most of them is the classification of herds according to the level of apparent seroprevalence measured with ELISA on meat juice samples collected at slaughter. The class of a herd is calculated as the weighted average of the most recent values of seroprevalence measured in batches delivered to the slaughterhouse over time. For each class above the first, for which usually there are no mitigation measures (Alban et al., 2002), the means for reducing *Salmonella* prevalence in the herd of origin are specific on-farm interventions (which may include the collection of fecal samples for examination, hygiene measures and movement restrictions) (European Food Safety Authority (Panel on Biological Hazards), 2006). Control programmes have been successful in countries like Denmark, where meat juice samples are collected for serology at slaughter, or Sweden, where lymph nodes are collected for bacteriology (Wegener et al., 2003). However, the control programme of the United Kingdom, which was also based on serology, gave mixed results and was suspended after ten years (British Pig Executive, 2012).

Currently, there is no national pig *Salmonella* control programme in Italy, where the *Salmonella* prevalence of slaughter pigs measured in lymph nodes was estimated to be 16.5% (95% CI 14.1–19.1; n = 709) according to an EFSA survey (European Food Safety Authority, 2008).

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Italian pig breeders are specialized in the 'heavy pig', which represents 90% of the Italian pig population. Heavy pig farming is mainly located in the Lombardy region (Northern Italy), and it comprises about 9000 herds of 1000 or more pigs (Banca Dati Nazionale, 2014). Heavy pig farming is characterized by a long production cycle with growth and finishing phases lasting for about 190–200 days so that pigs can meet the necessary weight (160–180 Kg) and fat-quality standards for the production of dry cured hams such as Prosciutto di Parma and Prosciutto di San Daniele. Because of their long life span, heavy pigs are exposed to the risk of becoming infected with *Salmonella* for a longer period than light pigs.

Salmonella seroprevalence in heavy pigs has not been estimated yet, although it would be important to have such information before initiating a control programme based on serology. The first objective of this study was to provide the estimation of the true herd *Salmonella* seroprevalence, the true within-herd seroprevalence and the true average seroprevalence in heavy pigs. The second objective was to investigate the association between the true status of infection and serological results to verify whether serology could identify infected individuals in this specific pig population.

2. Materials and methods

2.1. Study design and sample collection

Data used in this study were collected in the context of a research project for the characterization of the most common infectious agents affecting the Italian heavy pig. Sampling was conducted between January 2006 and February 2009 in a single slaughterhouse with capacity of over 450,000 heavy pigs per year, which represents about 5% of heavy pigs slaughtered in Northern Italy every year (Banca Dati Nazionale, 2014). A two stage cluster sampling protocol was adopted. In the first stage, batches of heavy pigs of herds located in Northern Italy were randomly selected from the beginning to the end of the work day, and in different days of the week. In the second stage, a blood sample was collected from one pig in every four on the slaughter line. The selection of the first pig was random, and 20 pigs were selected from each batch.

During the first six months of the sampling period (January–August 2006), cecal content and ileocecal lymph nodes (ICLN) were also taken from 19 batches randomly selected from six preselected large herds. Five herds were from the Lombardy region and one from the Veneto region. To avoid cross contamination, sterile gloves were changed after each sampling. Intestine packets were put in a single chilled plastic bag and processed at the laboratory within 24 h. At the laboratory, ICLN were immersed for 10 s in 95% ethanol and then passed through a flame to sterilize the surface and avoid cross contamination. Then, one sample of 25 g was taken from ICLN and another from the cecal content for examination. Samples of the same heavy pig (serum, ICLN and cecal content) were matched by a numerical identifier.

2.2. Serology

Serological examination was performed using a commercial indirect ELISA capable of detecting antibodies against lipopolysaccharide antigens of *Salmonella* serogroups B, C1, and D (HerdChek® Swine *Salmonella* Antibody Test Kit, IDEXX Laboratories, Liebefeld-Bern, Switzerland).

ELISA was performed according to the producer's instructions, and results were expressed in optical density percentage (OD%) of a known positive control.

2.3. Bacterial culture

Bacteriological examination was performed on a 25 gram portion and the procedure followed the ISO 6579: 2002/Amendment 1:2007 protocol.

Once isolated, strains were serotyped with O and H antisera according to the Kauffmann–White scheme (Grimont and Weill, 2007) at the Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna, Brescia, Italy. In case of isolation of *Salmonella typhimurium* or *Salmonella enteritidis*, strains were submitted for phage typing to the National Reference Laboratory for *Salmonella* at the Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy.

2.4. Datasets

For the first objective of the study (i.e. the estimation of the true *Salmonella* seroprevalence in slaughter heavy pigs, Section 2.5.1), a dataset with individual serological results from the first batch delivered by each herd during the sample collection was used.

For the second objective of the study (i.e. the evaluation of the association between the true status of infection and serological results, Section 2.5.2), a dataset was used that contained individual serological and bacteriological results of heavy pigs belonging to different batches delivered by six herds.

2.5. Statistical analysis

2.5.1. Estimation of the true Salmonella seroprevalence in heavy pigs

For the estimation of the true *Salmonella* seroprevalence, a pig was considered serologically positive when the antibody level detected by ELISA was equal or above 10 OD% (the cut-off suggested by the producer), 20 or 40 OD%. These specific cut-offs were chosen among those more commonly found in literature. Different cut-offs were chosen for two reasons: first, to verify how the estimated true seroprevalence changed by varying the cut-off point; second, to check how each cut-off changed the size of hypothetical seroprevalence classes as defined in the German *Salmonella* control programme. The German control programme (see details in the Discussion section) was selected because the data on the distribution of herds in seroprevalence classes was available in the period immediately preceding this study (European Food Safety Authority (Panel on Biological Hazards), 2006).

Seroprevalence estimation was carried out using the Bayesian latent class hierarchical model proposed by Branscum et al. (2004). According to this model, in the specific setting of this study, the number of positive serological results y in a single herd is assumed to be a series of independent Bernoulli trials and it follows the binomial distribution:

$$y|\pi, Se, Sp \sim Bin\,(n, \pi Se + (1-\pi)Sp))$$

where π is the within-herd *Salmonella* seroprevalence, Se is the sensitivity of ELISA, Sp the specificity, and n the number of pigs randomly sampled from that herd. Such assumption of the likelihood holds if the sample size (that is the number of pigs really tested) is very small compared to the herd size (that is the total number of pigs in the herd), because the sequence of Bernoulli trials is carried out without replacement (that is pigs were not reintroduced in the herd, and so the probability was not constant for each trial).

Being proportions, the Se and Sp are modeled as beta prior distributions. Also uncertainty around π can be modeled as a beta prior distribution, but with probability equal to τ, the herd seroprevalence, because if no pigs of the herd are seropositive, then π is zero:

$$\begin{aligned} &\pi \sim Beta\,(alpha_\pi, beta_\pi) \quad \text{with probability } \tau \\ &\pi = 0 \quad \text{with probability } (1-\tau)\,. \end{aligned}$$

In a multiple herd setting, all the π in the population of all herds can be thought as independent and identically distributed and to follow the beta distribution:

$$\pi \sim Beta\,(\mu\psi, (\psi(1-\mu))$$

where the hyper-parameter μ is the average seroprevalence of slaughter heavy pigs, and the hyper parameter ψ represents its variability across herds. Therefore π depends on μ and ψ.