Estimation of sensitivity and specificity of an indirect enzyme-linked immunosorbent assay (ELISA) for detection of antibodies against *Salmonella enterica* in meat juice and of microbiological examination of caecal content and mesenteric caecal lymph nodes for *S. enterica*

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**Abstract:** The aim of this study was to estimate the sensitivity and specificity of an indirect enzyme-linked immunosorbent assay (ELISA) for detection of antibodies against a variety of *Salmonella enterica* serovars in meat juice and of standard microbiological methods for detection of *S. enterica* in caecal content and in mesenteric caecal lymph nodes. Latent-class models were used for the estimation because no gold standard was available. The sensitivity of the ELISA was determined at different cut-off values of the optical density. The estimated sensitivity of the ELISA was 37%, 50% and 60% when the test was considered positive at an optical density ≥ 40, 20 and 10, respectively. Estimates of the sensitivity of the microbiological examination of caecal content ranged from 32% to 39% and of the mesenteric caecal lymph nodes from 27% to 33%. The estimated specificity of all diagnostic procedures was 100%.

**Keywords:** Diagnostic test validation; *Salmonella* surveillance;

**Introduction:** The nation-wide *S. enterica* surveillance and control program in Denmark (Mousing et al., 1997) is based on the monitoring of presence of antibodies against *Salmonella* in meat juice from carcases by an indirect ELISA (Nielsen et al., 1998). The ELISA includes several *S. enterica* O-antigens and detects a variety of *S. enterica* serovars. The sensitivity and specificity of the ELISA has only been determined in a small-scale experimental study (Nielsen et al., 1998). The quality of standard microbiological methods for detection of *S. enterica* in caecal content or in the mesenteric caecal lymph nodes has also been poorly documented. The aim of this study was to estimate the sensitivity and
specificity of the ELISA and of the microbiological methods for detection of S. enterica in caecal content and mesenteric caecal lymph nodes.

Materials and Methods: Data for this study was collected at 3 Danish slaughterhouses in 1999 (Sørensen et al., 2000). Herds were sampled from 8 strata based on the within-herd proportion of sero-positive pigs (0-0.1, 0.11-0.2, 0.21-0.3, 0.31-0.4, 0.41-0.5, 0.51-0.6, 0.61-0.7, 0.71-1). From each herd, samples of caecal content, caecal lymph nodes and meat were collected from 10 pigs. Individual samples of caecal content and lymph nodes were examined microbiologically according to the NMKL (Anon., 1991) including non-selective pre-enrichment, followed by selective enrichment, spreading on agar plates and serotyping. Individual meat samples were frozen and meat juice was harvested after thawing and examined by an indirect ELISA (Nielsen et al., 1998). A more detailed description of the sampling procedures is given by Sørensen et al. (2000). Latent-class models were used for the estimation of test sensitivity and specificity and their 95% confidence intervals (CI95) because no gold standard was available. The estimation was carried out by maximum likelihood (EM-algorithm) (Enøe et al., 2000). Individual samples were regarded as test positive in the microbiological examination if S. Typhimurium or any other S. enterica serovars were detected and in the ELISA if antibodies against S. Typhimurium or S. enterica serovars with O-antigens 1, 4, 5, 6, 7 or 12 were detected. Data was analyzed at 3 different cut-off values for the ELISA (test positive at an optical density % (OD%) ≥ 10, 20 and 40). Sufficient data for the estimation (tripled test results) was present from a total of 1,704 carcasses from 163 herds. The latent-class estimation was carried out using herd-level data to form populations.

Results: The estimated sensitivity of the ELISA was 36.8%, CI95: (30.2, 43.4), 50.2%, (43.9, 56.6) and 59.8%, (53.5, 66.2) when the test was considered positive at an OD% ≥ 40, 20 and 10, respectively. Estimates of the sensitivity of the microbiological method for detection of S. enterica in caecal content were 39.1%, (32.7, 45.4), 35.5%, (30.0, 40.9) and 32.0%, (27.0, 37.0) and estimates of the sensitivity of the microbiological method for detection of S. enterica in mesenteric caecal lymph nodes were 33.0%, (26.9, 38.8), 29.9%, (24.6, 35.1) and 26.9%, (22.4, 31.5) when the ELISA was considered positive at an OD% ≥ 40, 20 and 10, respectively. The estimated specificity of all diagnostic procedures was 100% in all analyses.

Discussion: Nielsen et al. (1998) reported estimates of the sensitivity of the ELISA for examination of meat juice that were considerably higher (between 80% and 100%) than our estimates. However, in the validation Nielsen et al. (1998) used an
ELISA for examination of serum as a gold standard. The two ELISA methods are basically identical and therefore the outcomes of the two tests are expected to be strongly correlated and this may have introduced some bias. The latent-class method does not have to rely on a gold standard. The latent-class models are based on the assumption that populations of truly diseased and non-diseased individuals exist although these populations are non-observable (i.e. latent). In the present study, the three diagnostic methods did not necessarily measure exactly the same disease or condition – the ELISA measured the presence of antibodies indicating whether a pig has been exposed to S. enterica in the past and the two microbiological methods measured a present (caecal lymph nodes) and a potential exposure (caecal content) to S. enterica. This is reflected in the slight differences in the sensitivity estimates of the two microbiological methods at different cut-off values of the ELISA. The estimated sensitivities of the diagnostic methods indicate that they far from always detect the presence of Salmonella. The estimated specificities suggest that false positive test results rarely occur. Furthermore, the results demonstrate the necessity of validating diagnostic procedures, especially when these make up the backbone of surveillance programs.

References


