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DETECTION OF YERSINIA ENTEROCOLITICA IN PORK

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Introduction:

During recent years, there has been a measurable increase in *Yersinia enterocolitica*, which belongs to the enterobacteriaceae family, as the cause of human infections through the consumption of contaminated meat and meat products. This is in spite of the low intensity of investigation for this agent of zooanthroponoses. *Yersinia enterocolitica* possesses characteristics which favour survival and growth in foods of animal origin. These include an optimal growth temperature of 28°C, relative osmotolerance, reduced nutritional requirements, as well as growth in a moderate table salt environment. Of particular significance is the psychrotolerance of *Yersinia enterocolitica*, which is capable of reproduction even at 4°C, thus it poses a risk in refrigerated foods such as fresh meat, poultry, and milk products. These characteristics give *Yersinia enterocolitica* the capacity to survive under unfavourable conditions. For its successful bacteriological detection, it is necessary to apply suitable methods for the rapid and specific diagnosis of this disease agent. The difficulty of reliably isolating *Yersinia enterocolitica* is evident both from older (LEISTNER et al., 1975) as well as from more recent literature (KARIB et al., 1994). After BÜLTE et al. (1991), the incidence of *Yersinia enterocolitica* isolates in pork is 4%. There is a potential for carcasses to become contaminated during slaughter at many points during the slaughter process, e.g. during evisceration, removal of tonsils, etc. (BORCH et al., 1996).

Materials and methods:

From March 1997 to November 1997, contamination with *Yersinia* was investigated in 540 samples taken at two slaughter houses, A and B, in north-western Lower Saxony, from 260 slaughtered pigs which had already passed the routine official inspection procedure without objection. The samples consisted of the following: 260 tonsil samples, 160 tongue samples and 30 samples each from abdominal and neck musculature, mesenterial lymphnodes, and caecum content, respectively. Samples were taken once a week under sterile conditions from slaughter pigs immediately after evisceration directly at the conveyor belt. Transport was at 2-4°C and took place immediately after being obtained, when they had been packaged and sealed in isolated cool bags.

Results:

Using the enriching broth after OSSMER, it was not possible to isolate *Yersinia* in 150 of the samples.Using the ISO technique (1994) as a guideline for detecting presumptive *Yersinia enterocolitica* strains, it was also not possible to detect *Yersinia* in 320 samples. In the later course of the investigations, the ISO technique was scrutinized in 70 samples for its comparative suitability for detecting *Yersinia enterocolitica*. No *Yersinia* could be detected. In 15 (21.4%) tonsil samples, it was possible to detect *Yersinia enterocolitica* with cultures using cold enrichment. During pre-enrichment in the first week, it was possible to demonstrate *Yersinia enterocolitica* in 13 tonsils. One more *Yersinia enterocolitica* detection was made during each of the second and third weeks of pre-enrichment.11 samples which were positive for *Yersinia* after cold enrichment were also positive for *Yersinia* in the two-step-enrichment and after incubation at 4°C. With the aid of cold enrichment, it was possible to detect *Yersinia enterocolitica* in a total of 21 samples (30%). During the first week of pre-enrichment, 16 tonsils were positive, in the second week further 3 tonsils, and in the

third week two more tonsils were *Yersinia*-positive. In the course of biochemical and serological differentiation, the following serotypes were confirmed: 20 isolates of *Yersinia enterocolitica* serotype 0:3/biotype 4; two isolates of *Yersinia enterocolitica* serotype 0:6/biotype 1A; one isolate of *Yersinia enterocolitica* serotype 0:41,43/biotype 1a; two isolates of *Yersinia* intermediate.

Discussion:

The detection of *Yersinia enterocolitica* in foods and meat is difficult, since, depending on the sample material involved, this microbe is usually outnumbered by the accompanying flora, and can thus only be diagnosed in culture under very specific conditions. In the present paper, various culture methods were tested for their suitability for diagnosing this disease agent in slaughter pigs.

Yersinia-selective enrichment broth after OSSMER: In our investigations, no *Yersinia* could be detected in 150 samples. One reason for this may have been that the recommended incubation temperature of 30°C is too high, or that the concentrations of irgasan and bacitracin had an effect on the growth of *Yersinia enterocolitica*.

ISO technique (ISO/DIS 10273, 1994): Neither modified selenite-broth nor two-step enrichment procedure (pre-enrichment with yeast extract Bengal red broth; enrichment with gall oxalate sorbose broth) proved suitable for isolating *Yersinia enterocolitica*.

Cold enrichment: Cold enrichment (MAIR and FOX, 1986) is a commonly used means of detection. In this procedure, advantage is taken of the capacity of *Yersinia enterocolitica* to grow even at 4°C. Our comparative study showed that, in addition to the nature, the quantity and the combinations of various ingredients with a selective capacity, the incubation temperature had a marked influence on the rate of detection of *Yersinia enterocolitica*.

Two-step cold enrichment: In 21 (30%) of tonsil samples, it was possible to detect *Yersinia enterocolitica* using the two-step cold enrichment. Of the 21 *Yersinia*-positive samples, 18 (85.7%) were identified as pathogenic *Yersinia enterocolitica* serotype 0:3. The first medium in the two-step enrichment, i.e. PBSSB, serves to allow the growth of a small or damaged number of *Yersinia* cells at 4°C for 3 weeks. The second medium, i.e. modified RAPPAPORT medium, has a selective function which facilitates the later isolation of suspected colonies.

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