THE ISOLATION PREVALENCE OF BACTERIA BELONGING TO YERSINIA GENUS ISOLATED FROM CARCASSES AND ORGANS OF WILD BOAR

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Summary

This study follows the isolation frequency of Yersinia germs from different organs and carcasses samples collected from wild boars. The study presents a high importance because it reveals the probability of contamination with bacterial species of Yersinia genus, which are pathogen for human beings (especially for infants) through alimentary toxic infections, manifested by austere diarrhea syndrome.

To determine the isolation prevalence of Yersinia bacteria, a total number of 4905 organs and muscle sample were harvested and processed through microbiological assays; the study was developed during a period of 5 years (2003-2007).

The statistical analysis of the data obtained showed a total prevalence of 2.26 with a yearly fluctuation of frequency isolation situated between short limits (from 1.02 % to 0.1 %).

The epidemiological studies performed by groups of scientists from all over the world concluded that the bacterial species comprised in Yersinia genus are pathogen only for humans and primates. Many epidemical episodes registered in countries with a developed sanitary system and a elevated hygiene level, captivated the attention by an exalted number of clinical cases and the severe morphological and clinical signs. However, the actual real rate of morbidity by toxic infection with Yersinia is higher than the official one, the increasing number of episodes all over the world generating a serious reason of concern for public health.

The origin of food borne diseases with Yersinia are a series of vegetal food products (salads, fruits, green vegetables) or mixed products (salad with meat or cheese), the animal products being rarely incriminated (especially milk and milk products are considered risk products). Habitually the animal products are contaminated while non-hygienically processing stages or through their manipulation by infected persons or chronic wearers. A series of studies demonstrated even the possibility of Yersinia germs’ multiplication on contaminated food products with a minimal germs’ content, the multiplication taking place even at 10°C (13, 14).

Taking into account the deficient hygiene conditions assured while evisceration and transport of game (wild boars), we considered propitious the microbiological investigations in order to statistically determine the isolation
frequency of Yersinia germs, a motivation being the habit of this species to frequent forests with still water sources (swamps, marshlands) which are constantly contaminated with faecal germs.

**Material and methods**

In order to establish the isolation prevalence of Yersinia germs, there have been collected and bacteriological analyzed samples of muscle from different anatomical parts of wild boar carcasses, sanitary swabs loaded on peritoneal surfaces and parietal pleura and also different portions of organs (liver, spleen, kidney, mesenteric lymphatic centers, and intestinal portions).

The collection of the samples was performed mainly from recently hunted wild boars (maximum 60 minutes after the blast), obtained by authorized hunting in small groups or collective hunting.

The area comprised in this survey is the hunting areas from the southern and south-eastern part of the country, including the region of the Danube Delta. The sample collection was done often by the authors themselves; yet, taking into consideration the restrictions of wild boar hunting, some of the samples have been collected by the hunters, especially prepared to perform this operation.

The sample collection was executed between 200 and 2007 and gathered a total number of 4905 samples. The number of samples processed annually and the modality of their presentation are described in table 1.

**The identification of sample types collected annually and processed through bacteriological techniques**

<table>
<thead>
<tr>
<th>Type of sample</th>
<th>Number of samples collected by entire period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2003</td>
</tr>
<tr>
<td>Samples of muscle from carcass</td>
<td>156</td>
</tr>
<tr>
<td>Samples of liver portions</td>
<td>96</td>
</tr>
<tr>
<td>Samples of spleen portions</td>
<td>96</td>
</tr>
<tr>
<td>Samples of intestinal portions</td>
<td>107</td>
</tr>
<tr>
<td>Samples of mesenteric lymphatic centers</td>
<td>107</td>
</tr>
<tr>
<td>Samples of kidney</td>
<td>96</td>
</tr>
<tr>
<td>Sanitary swabs</td>
<td>115</td>
</tr>
<tr>
<td>Total samples</td>
<td>773</td>
</tr>
</tbody>
</table>

The sample laboratory processing followed also the work stages comprised in the ISO techniques (SR ISO 10,273, SR 12925, STAS 2356-82), and also the work techniques used actually by different microbiology laboratories in order to
establish a diagnosis of this bacterial species. The work method used in this case comprised several stages:

- the collection of the samples from animal food products is realized in strict aseptic conditions. From each sample a quantity of 10 g of muscle tissue is taken, for meat samples or, depending on the situation, 10 ml of milk or milk product, which are introduces in the low enrichment media, obtaining a percentage of 1/10 or 1/100 (percentage mass/volume or volume/volume).

- the enrichment is realized in 2 different selective liquid media: 10 g (ml) of sample are introduced in 90 ml of PSB medium (peptone-sorbitol-biliar salts broth), obtaining a percentage of 1/10. The incubation is realized at 25°C for 5 days. Another 0.1 g (ml) of sample are introduced in 9 ml of ITC medium (irgasan-ticarclylin-potassium chlorate broth), obtaining a percentage of 1/100. The incubation is realized 25°C for 2 days.

- for isolation and identification, a culture loop from the enrichment broth PSB is passed directly on the surface of CIN agar and 0,5 ml of culture from the same medium is transferred in 4,5 ml of KOH saline solution 0,25 % and is homogenized for 10-20 seconds. After the homogenization a culture loop is passed on the CIN agar surface.

In parallel, a loop from the culture obtained in the enrichment broth ITC is passed on the surface of SSDC gelose (Salmonella-Shigella, with sodium desoxycholate and calcium chloride). The incubation of the dishes is realized at 30°C, in aerobiosis (?), for 24 h. After 24 hours, the dishes are examined with a magnifying glass or using an oblique light to recognize the characteristic colonies of Yersinia enterocolitica. On the surface of CIN gelose, the characteristic colonies are small (1 mm), flat, with a red spot in the center, surrounded by a pink area, semitransparent (having the aspect of an “ox’s eye”), and bile precipitate, and, after examining on oblique light, they are tiny granulated and not iridescent. On Wauters (SSDC) gelose, the characteristic colonies are small (1 mm) and grey, with a thin margin, and by examining on oblique light, they are tiny granulated and not iridescent.

For confirmation, a number of 5 characteristic and/or suspect colonies are selected from the selective culture media dishes, which were reintroduced on the surface of some dishes with nutritive gelose in order to obtain pure cultures to perform the confirmation through biochemical assays. The dishes with sowed nutritive agar are incubated at 30°C for 24 h.

The identification-confirmation stage was realized by using API 20E galleries: the API 20E system offers the possibility of identification of Yersinia germs in 24 h, as well as other enteric bacteria. The gallery presents 20 microtubes which contain dehydrated substrates for ascertaining of enzymes, carbohydrates’ assimilation or inhibition assays. The gallery comprises 2 parts. The first one contain the enzymatic tests and the conventional ones. The dehydrated substrates were inoculated with a suspension of germs which helps rehydrating these ones. The manifested reactions during the incubation period (at 37°C in aerobiosis) were
spontaneous color modifications or the ascertaining of the color after adding some specific reagents. The second part of the gallery comprises the assimilation or inhibition tests. After incubation at 37°C for 24 h, the reactions were read visually, using the table of reading and identification or the identification catalogue API 20E.

The gallery can offer and realize: enzymatic reaction which ascertains the presence of beta-galactosidase (ONPG); 5 classical chemical reactions: urease (URE); the reduction of nitrates in nitrites (NO₂); the production of H₂S (sulphuric hydrogen); the production of indole (IND), the production of acetoin (VP); 10 assimilation reactions: the assimilation of glucose (GLU); the assimilation of mannitol (MAN); the assimilation of inositol (INO); the assimilation of sorbitol (SOR); the assimilation of rhamnose (RHA); the assimilation of saccharose (SAC); the assimilation of melibiose (MEL); the assimilation of amygdaline (AMY); the assimilation of arabinose (ARA); the assimilation of citrate; an enzymatic reaction which ascertains the gelitinase (GEL); an enzymatic reaction which ascertains the dehydrolase: arginin-dehydrolase (ADH); 2 enzymatic reaction which ascertain the decarboxylase: lysine-decarboxylase (LDC); ornithin-decarboxylase (ODC); an enzymatic reaction for ascertaining cytochrome-oxidase (OX); an enzymatic reaction for ascertaining tryptophane-desaminase (TDA). The API 20E Gallery, BIO MERIEUX S.A. comprise: 25 API 20E galleries; 25 incubation dishes; 25 result sheets; 1 technical sheet; TDA reagent; IND reagent; VP1 and VP2 reagents; NIT1 and NIT2 reagents; OX reagent; paraffin oil; pipettes. Initially a Gram coloration is performed on every analyzed stems and, afterwards, the reactions for betagalactosidase, urease and H₂S, in order to examine the inherence of the germ to Yersinia enterocolitica species. A dish with CIN agar is prepared; the dishes are incubated at 30°C for 24-48 hours; it is verified the presence on the slide of Gram-negative cocobacilli, bipolar dyed; a culture from the selective isolation media dish is collected using a sterile swab (carefully, in order to avoid the penetrating of the medium) and a suspension in 3 ml of NaOH 0.85% is realized, bringing the suspension at a turbidity equal to 6 on McFarland scale. The dehydrated substrates of the gallery are inoculated with the bacterial suspension, and the incubation is realized at 37°C, in aerobiosis. At this moment, the interpretation of the results is done, using the reading table of the gallery.

Results and discussions

The statistical analysis of the performed research on a total number of 4905 samples permitted the conclusion of an annual incidence at relatively low values, with variations from a year to another. The number of positive samples and the isolation frequency of Yersinia germs are synthetically presented in table 2.

The results obtained demonstrated a low incidence at portage of bacteria belonging to Yersinia genus (maximum 2.34 %), but these low values can be determined by the inhibition of Yersinia bacteria by preferential development of other bacteria which populate the intestine. The highest isolation frequency was
observed at the level of serous surfaces (pleura and peritoneum), this specific observation demonstrating the carcass post-mortem contamination either because of wild boar evisceration in improper conditions (evisceration in improper conditions and minimal techniques) or because of the carcass faecal contamination.

There haven’t been observed isolations from parenchimal organs, and from the muscle samples there has been made a single isolation (which we can consider accidental), the Yersinia germs which accede to the organism through contamination by oral way, arrive at the mesenteric lymphatic center as maximum level.

Table 2

<table>
<thead>
<tr>
<th>The type of samples</th>
<th>The number of positive samples and the isolation frequency (%) / period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2003 no. / %</td>
</tr>
<tr>
<td>Samples of muscle from carcass</td>
<td>0 0 1</td>
</tr>
<tr>
<td>Samples of liver portions</td>
<td>0 0 0</td>
</tr>
<tr>
<td>Samples of spleen portions</td>
<td>0 0 0</td>
</tr>
<tr>
<td>Samples of intestinal portions</td>
<td>2 1.87 3</td>
</tr>
<tr>
<td>Samples of mesenteric lymphatic centers</td>
<td>1 0.93 1</td>
</tr>
<tr>
<td>Samples of kidney</td>
<td>0 0 0</td>
</tr>
<tr>
<td>Sanitary swabs</td>
<td>3 2.61 5</td>
</tr>
<tr>
<td>Total samples</td>
<td>6 0.78 10</td>
</tr>
</tbody>
</table>

Although the total prevalence, in the survey period, demonstrates low values (0.49), it can be concluded the possibility of these bacteria to contaminate the wild boar carcasses, these constituting contamination sources for consumers. Following this, a series of alimentary products obtained through thermal processing by using methods which involve low values of temperature applied on short periods of time, or alimentary products consumed without being processed (raw), can be involved in alimentary toxic infections episodes.
Conclusions

1. After collection and processing through techniques and bacteriological assays a total number of 4905 samples of muscle, different organ parts and sanitary swabs pleura and peritoneum, there have been isolated a number of 24 bacterial stems which were identified as belonging to the species included in Yersinia genus.

2. The statistical analysis of the obtained results on a 5 years period permitted the establishing of a total prevalence of 0.49. The annual variations of the isolation frequency were situated between relatively low values (between 1.02% and 0.1%).

3. From the total number of analyzed sample types, the highest value of isolation frequency was observed for the sanitary swabs of pleura and peritoneum (1.96% for the total period), the other isolations being considered accidental.

4. The bacterial species form Yersinia genus can contaminate the wild boar carcasses, the products and by-products obtained after processing, these carcasses being considered the origin of food toxic infections.

References


