The Prevalence of *Yersinia enterocolitica* Species in the Flow of Butchering

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Abstract
During the experimental stages, the researches aimed to establish the prevalence of *Y. enterocolitica* bacteria on swine, in the butchering flow. The experiments developed on a large number of test specimens (800), sampled starting with the moment of animals receiving and until the final product was obtained.
For isolation and identification there were used a modified method, proposed by The International Organization for Standardization, and CIN and SSDC isolating cultures as well. Following the effectuated researches, in accordance with the international ones, we can conclude that, in the present, the butchering process allows the strict observance of the hygiene and disinfection conditions with the purpose of limiting the dispersion of *Yersinia enterocolitica*, which favors the phenomenon of inter-contamination.

Keywords: alimentary toxic infections, identification, isolation, *Yersinia enterocolitica*.

1. Introduction

The incidence of alimentary toxic infections having as etiologic agent *Yersinia enterocolitica* is far from being known because there weren't reported all the cases. The number of confirmed cases of toxic infections with *Yersinia enterocolitica*, differs from one country to another, depending on the preoccupations of the specialists and on the isolation and identification possibilities of the laboratories [1,2,3,4]. During the processes of butchering and swine meat processing a contamination with microorganisms can take place, which can depreciate the nutritive value of the final products and, in some cases, can produce the sicken of the consumers [4,5,6].
Considering theses aspects, the research of *Yersinia enterocolitica* presence during the process of butchering and swine meat processing was considered important and opportune. The basic objective during the study was to establish the prevalence of *Yersinia enterocolitica* within the species of swine, from the start being imposed to us the verification of three major rings until the final product is obtained (from the entrance in the butchery, during the technological flow of processing and in the commercial chain). Following our researches, in accordance with the international ones, it results that, in the present, the butchering process allows the strict observance of the hygiene and disinfection conditions with the purpose of limiting the dispersion of *Yersinia enterocolitica*, which favors the phenomenon of inter-contamination [7,8,9].

2. Materials and methods

For the evaluation of the incidence of *Yersinia enterocolitica* species within the swine species there were sampled and analyzed 800 test specimens from two units A and B, aiming the verification of 16 lots of ten animals (eight groups from unit A and eight groups from unit B) during the technological flow of butchering. The sampling points of the tests were represented by: swine receiving (160 test specimens), evisceration (160 test specimens) of which, organs (liver, kidneys and spleen of 40 test

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specimens), carcass (40 test specimens), refrigeration (160 test specimens) and carcass congealing (160 test specimens).

For the isolation and identification of *Yersinia enterocolitica* presence it was used a modified method proposed by The International Organization for Standardization ISO/DIS 10273/1994. The test specimens sampled from the *intestinal* content were inoculated directly on the surface of the environment with the remark that on each Petri plate were striated 3 test specimens. In parallel the CIN and SSDC (Wauters) environments were inoculated. The incubation was made in thermostat at a temperature of 30ºC for 24-48 hours. The same test specimens were diluted in 10 ml of normal saline solution and, after the dilution and homogenization, 1 ml of this slurry was inserted in 9 ml of KOH solution 0.25% and 0.85% NaCl. After an agitation of 10 seconds the content of a loop 0.1 ml there were inoculated on the surface of the selective isolating environment (CIN, SSDC and Modified). The inoculated plates were incubated for 24-48 hours at the temperature of 30ºC, under aerobe conditions.

3. Results and discussion

The study of the carriage before butchering

In order to isolate the germs of *Yersinia enterocolitica* of intestinal origin there were sampled 160 test specimens, sampled with the help of swabs from the rectum, from two units A and B, aiming the verification of 16 lots of 10 animals (8 lots from unit A – 80 samples and 8 lots from unit B – 80 samples).

The sampling of the test specimens was made after the classification in quality classes (depending on age, sex, weight, conformation, fattening status, etc.), from pig lots that were in bad shape, with diarrhea (generally aqueous, not sanies), as well as from animals which at the sanitary veterinary check before slaughter were in a good shape.

After the sampling, the swabs were inserted in test tubes with phosphate-sorbitol-gallbladder salts environment (PSB), in a quantity of 5 ml, after which they were inoculated on culture environments for isolation (CIIN and SSDC).

Of the 160 samplings (rectal swabs), by direct inoculation following the bacteriologic examination for the identification of the *Yersinia enterocolitica* species there were identified 5 positive test specimens, 3 of unit A and 2 of unit B (Table 1).

Table 1. Overview of the results of bacteriological analysis, *Yersinia enterocolitica* home intestinal before slaughter in units A and B (direct isolation)

Unit	Lots No.	Analyzed test specimens No.	Of which positive			
			Lots		Test specimens	
			No.	%	No.	%
A	8	80	3	37.50	3	3.75
B	8	80	2	25.00	2	2.50
A+B	16	160	5	31.25	5	3.125

The obtained data stresses a higher incidence in unit A that was determined by the different provenience of the animals (Figure 1).

The positive test specimens were put under biochemical testing for the confirmation and identification of *Y. enterocolitica*. The results of the biochemical tests stressed that the five isolated strains belong to the *Y. enterocolitica* species (Table 2).

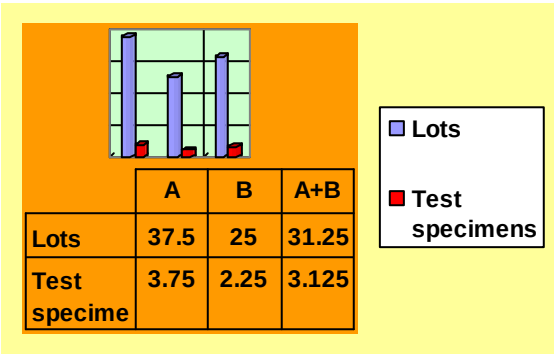

Figure 1. The dynamic of carriage before slaughtering

Table 2. Results of bacteriological analysis for *Yersinia enterocolitica* of intestinal origin before slaughter in units A and B (prior enrichment of samples)

TEST	STRAIN				
	1	2	3	4	5
Ornithine decarboxilaxa	+	+	+	+	+
Urease	+	+	+	+	+
Glucose acid	+	+	+	+	+
Oxidase	-	-	-	-	-
Lysine decarboxilaxa	-	-	-	-	-
Rhamnose –	-	-	+	-	-
Citrate	-	-	-	-	-
Saccharose	+	+	+	+	+
Hydrogen sulfurate	-	-	-	-	-

The study of *Y. enterocolitica* incidence after slaughtering

The technological process of slaughtering starts with electrical stunning and the bleeding of swine, complete scalding in a tank with hot water, approximately 2 minutes, after which they are hanged on the conveyor line.

In the two studied slaughter houses, the carcasses were brought by conveyors, after singeing and washing, in front of the evisceration band, provided with fixed trays in which the organs are putted into during the evisceration.

The operation of evisceration is made by a circumanal and abdominal incision after which the gastro intestinal mass is extracted. The spleen is detached from the gastro intestinal mass and it is processed in a separate compartment. Afterwards, the stern is cut with the help of electrical saw.

The evisceration technique of swine supposes the extraction in a common piece of multiple organs, such as: the tongue, the esophagus, the trachea, the heart and the liver. The kidneys remain in natural adherence and are extracted together with the perirenal adipose tissue. The sectioning of carcasses in two halves is made with the mechanical saw.

We mention that after the evisceration were sampled test specimens from the intestinal and organ content (liver, kidneys, tongue and carcasses) that were inoculated on the selective isolating environments (CIN; SSTC and modified environment).

The obtained results at the bacteriologic test of the 160 test specimens sampled from the intestinal content of the animals from the 16 lots stressed and incidence of *Y. enterocolitica* of 8.125% but different depending on the used selective environment. Thus, the largest number of positive test specimens following the bacteriological test was ascertained in the case of modified environment utilization. If on the selective environments CIN and SSTC were identified 11 positive animals, on the modified environment was identified the presence of *Y. enterocolitica* at 13 animals, with the remark that the positive test specimens came from the animals of two lots of ten animals each unit (Table 3).

Table 3. The bacteriological analysis results of *Yersinia enterocolitica* germs within the evisceration operation in units A and B

Culture environment	Unit						Total		
	A			B			Analyzed test specimens No.	Of which positive	
	Analyzed test specimens No.	Of which positive		Analyzed test specimens No.	Of which positive			No.	%
		No.	%		No.	%			
CIN	80	7	8.75	80	4	5.00	160	11	6.875
SSTC	80	7	8.75	80	4	5.00	160	11	6.875
modified	80	8	10.00	80	5	6.25	160	13	8.125

Likewise, it was ascertained a different incidence of the presence of positive test specimens from one unit to another, but also depending on the used selective environment (Figure 2).

Following the testing of the biochemical activity of the 13 isolated strains from the intestinal content it was ascertained that they all belong to *Y. enterocolitica* (Table 4).

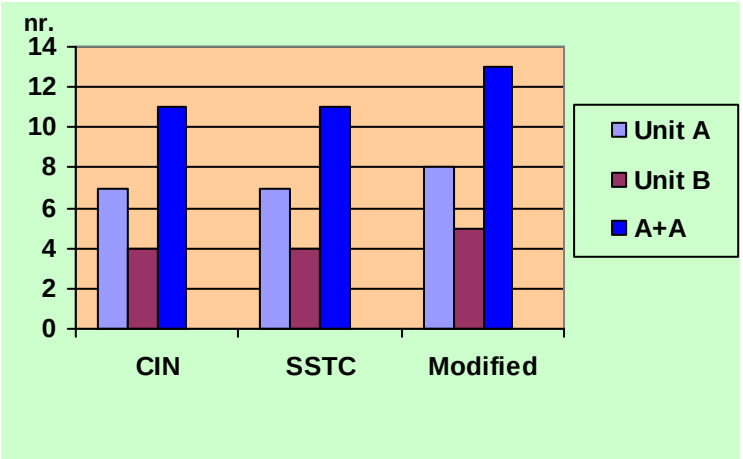

Figure 2. The incidence of positive test specimens within the operation of evisceration in units A and B

Table 4. Bacteriological analysis results of Yersinia enterocolitica germs within the evisceration operation in units A and B

Strain (from test specimen)	Test								
	Ornithine decarboxilaxa	Urease	Glucose acid	Oxidase	Lysine decarbo-xilaxa	Rhamnose	Citrate	Mobility at 25°C	Esculin
1	+	+	+	-	-	-	-	+	-
2	+	+	+	-	-	-	-	+	-
3	+	+	+	-	-	-	-	+	-
4	+	+	+	-	-	-	-	+	-
5	+	+	+	-	-	-	-	+	-
6	+	+	+	-	-	-	-	+	-
7	+	+	+	-	-	+	-	+	-
8	+	+	+	-	-	-	-	+	-
9	+	+	+	-	-	+	-	+	-
10	+	+	+	-	-	-	-	+	-
11	+	+	+	-	-	-	-	+	-
12	+	+	+	-	-	-	-	+	-
13	+	+	+	-	-	-	-	+	-

From the animals of the lots identified as positive at the testing of the intestinal content there were sampled 160 test specimens, 40 from liver, 40 from tongue, 40 from kidneys and 40 test specimens of sanitation sampled by wiping a gauged surface of 10 sq cm from the carcass.

The obtained results stressed a reduced incidence of the presence of *Y. enterocolitica* species at the level of the analyzed organs (0.83%). We mention that of the analyzed organs it was identified a single strain at the level of the tongue (Table 5).

Table 5. Number of positive samples of Yersinia enterocolitica germs isolated from organs after sowing directly on selective media

Organ	Unit						Total		
	A			B			Analyzed test specimens No.	Of which positive	
	Analyzed test specimens No.	Of which positive		Analyzed test specimens No.	Of which positive			No.	%
		No.	%		No.	%			
Liver	20	-	-	20	-	-	40	-	-
Tongue	20	1	5	20	-	-	40	1	2.5
Kidneys	20	-	-	20	-	-	40	-	-
Carcasses	20	3	10	20	1	5	40	4	10,0
TOTAL	80	4	5.0	80	2	1.25	160	5	3.125

Test specimen samplings at refrigeration and after the storage of refrigerated meat

In the purpose of qualitative evaluation of the contamination of carcasses belonging to the animals of the four lots identified as positive there were sampled 40 test specimens before refrigeration and 40 after refrigeration. The obtained results stressed that the refrigeration has negatively influenced the viability of *Y. enterocolitica* strain. Thus, the incidence at the level of the carcasses identified as positive is reduced from 5% to 3.75% (Table 6).

Test specimen samplings after refrigeration

The effect of low temperatures on the viability of *Y. enterocolitica* strains was stressed also in the process of carcasses freezing. Thus, after 24 hours since freezing the incidence of viable *Y. enterocolitica* strains is reduced from 5% to 2.5% and after 72 hours to 1.25% (Table 7).

Table 6. The obtained results after direct isolation of germ *Yersinia enterocolitica* after refrigeration and after the storage of refrigerated meat

Unit	The period of test specimen sampling					
	Before refrigeration			After refrigeration		
	Carcasses No.	Of which positive		Carcasses No.	Of which positive	
		No.	%		No.	%
A	40	1	2.50	40	1	2.50
B	40	3	7.50	40	2	5.00
Total	80	4	5.00	80	3	3.75

Table 7. Qualitative evaluation Yersinia enterocolitica contamination during freezing by direct isolation

Unit	The period of test specimen sampling					
	After 24 hours of freezing			After 72 hours of freezing		
	Carcasses No.	Of which positive		Carcasses No.	Of which positive	
		No.	%		No.	%
A	40	1	2,50	40	-	-
B	40	2	2.50	40	1	2.50
Total	80	3	2.500	80	1	1.25

4. Conclusions

During butchering, in the first stages there can be noticed a decrease of germs of the *Y. enterocolitica* species presence.

Following our researches, in accordance with the international ones, it results that, in the present, the butchering process allows the strict observance of the hygiene and disinfection conditions with the purpose of limiting the dispersion of *Yersinia enterocolitica*, which favors the phenomenon of inter-contamination.

Swine carcasses have shown following the qualitative evaluation a reduced contamination after direct isolation and after prerequisite enrichment.

Swine carcasses have shown by direct isolation a contamination of 10% after 24 hours since

refrigeration and 7.5% after 72 hours since refrigeration.

Within the evisceration operation, the swine organs have shown by prerequisite enrichment a reduced rate of contamination (contaminated lots after the isolation of *Yersinia enterocolitica* from rectal samples) for the test specimens from the liver, as well as spleen test specimens, kidneys and tongue test specimens.

It was noticed that when the evisceration is made, the contamination can be effectuated accidentally by the rupture of intestines, favoring the presence of the germ on the flow but in our case the butchering mistakes were sanctioned with complete confiscation.

The analyzed meat products have shown zero contamination level by direct isolation and by prerequisite enrichment as well.

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