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Relevance of Meat Juice Seroprevalence and Presence of *Yersinia enterocolitica* and *Salmonella* spp. in Pig Tonsils for Risk Management at Slaughter

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Abstract: *Salmonella* spp. and *Yersinia enterocolitica* are priority bacteriological public health hazards in pork safety. For more successful control, it is necessary to collect data on their prevalence throughout the meat chain using the concept of harmonized epidemiological indicators. The aim of this study was to determine their prevalence in fattening pigs under different housing conditions by recovering the pathogen from tonsils and by serological testing of diaphragm meat juice at slaughterhouses. The overall prevalence of *Salmonella* spp. and *Y. enterocolitica* in tonsils was 9.8% and 6.5%, respectively, with no significant differences between large and small farms ($p > 0.05$). In general, seroprevalence of *Salmonella* spp. was 48.35% and of *Yersinia* 13.18% ($p < 0.05$) but without significant differences of individual seroprevalence between farm types. No association was found between detection of *Salmonella* spp. or *Y. enterocolitica* in tonsils and seroprevalence ($\varphi c = 0.121$, $p = 0.420$; $\varphi c = 0.027$, $p = 0.718$, respectively). Significantly higher seroprevalence of *Salmonella* spp. was found on farms with lower biosecurity status ($p < 0.05$). A higher recovery rate of *Salmonella* spp. and *Y. enterocolitica* from the tonsils may be expected in seropositive pigs (OR 1.56–2.36), but without statistical significance. The results showed that *Salmonella* and *Yersinia* meat juice serology can be considered for risk categorization of pig farms as a less-time consuming and more sensitive method compared to microbiological testing of tonsils but must be combined with analyses of other risk factors relevant to infection or contamination in the pork chain.

Keywords: *Salmonella* spp.; *Yersinia enterocolitica*; seroprevalence; tonsils; pigs; farming systems

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Citation: Kiš, M.; Fuštin, D.; Zdolec, N. Relevance of Meat Juice Seroprevalence and Presence of *Yersinia enterocolitica* and *Salmonella* spp. in Pig Tonsils for Risk Management at Slaughter. *Processes* **2023**, *11*, 2234. https://doi.org/10.3390/pr11082234

Academic Editor: Chi-Fai Chau

Received: 25 June 2023
Revised: 14 July 2023
Accepted: 24 July 2023
Published: 25 July 2023

1. Introduction

There is no doubt that traditional meat inspection has made an important contribution to the protection of public health in the last century. However, the main drawback of such an approach, based on visual inspection with palpation and incision of meat and organs, is its limited ability to detect the main biological threats. According to the risk analysis conducted by the European Food Safety Authority (EFSA), the most important biological hazards in pork production at the farm and slaughterhouse level are the bacteria *Salmonella* spp. and *Yersinia enterocolitica* and the parasites *Trichinella* spp. and *Toxoplasma gondii* [1]. The challenge in listed hazards control in the meat production chain is their presence in latently infected, asymptomatic animals [2]. Consequently, infection with these pathogens in pigs does not result in visible or palpable pathological changes in the pig carcass, which is the most important reason for the inability of conventional meat inspection to detect them and effectively control the existing meat safety risks [3–5].

The European Food Safety Authority (EFSA) proposed the concept of Harmonized Epidemiological Indicators (HEIs) more than ten years ago [6] and defined them as "prevalence or concentration of the hazard at a certain stage of the food chain or an indirect indicator of the hazards that correlates to human health risk caused by the hazard". In

terms of farms, use of HEIs allows their risk categorization according to their risk exposure and their ability to control and reduce that risk at the abattoir level [7]. The fact that salmonellosis and yersiniosis are among the top three zoonotic diseases in Europe [8] indicates that risk mitigation strategies should be improved in pork production from farm to slaughterhouse. Considering both *Salmonella* and *Yersinia enterocolitica,* the testing of seroprevalence is not included in herd/farm risk categorization, but only the presence of pathogen in feces and/or tonsils [1]. Nevertheless, serological testing of meat juices is used as a method for classifying pig herds according to risk for *Salmonella* spp. in numerous national control programs in the European Union, such as the Danish *Salmonella enterica* control program, the German Q-S (Quality and Safety) system, and the Finnish *Salmonella* spp. control program, which has nearly eliminated infection [9]. The method is recognized as reliable, rapid, easy to perform, and cost-effective for the monitoring and control of *Salmonella* spp. in pig herds [10]. In addition, data on the seroprevalence of *Salmonella* spp. can be very helpful in determining the correct order of logistic slaughter and contribute to the efficient prevention of cross-contamination of carcasses at the slaughterhouse [11].

The results of the evaluation of serological test kits recorded by Meemken et al. [3] show that serological profiling and classification of pig herds into a "zoonotic risk" category and a "health risk" category can be of great help in risk-based decision making. Therefore, the development of a cost-effective test system for the simultaneous detection of different antibodies has been proposed for the large-scale implementation of the multiserological approach for meat juice [3]. In that respect, several studies have been conducted using meat juice serology in slaughter pigs for *Yersinia* spp. showing the high within-farm and farm-level seroprevalence [12–14].

In addition to seroprevalence (i.e., positive immunological response to antigens), the actual presence of the pathogen (*Salmonella* or *Y. enterocolitica*) on the carcass or in/on organs at slaughter must also be considered as an indicator of risk. In regard to that, to gain insights into the reliability of serological monitoring and within connected measures at harvest level, it is important to compare the results of seroprevalence with microbiological findings in organs, considering the fact that the carriage of *Salmonella* and *Y. enterocolitica* in the tonsils has been recognized as a risk factor for meat contamination during slaughter [15,16]. Possible discrepancies in results and their proportions, especially in the case of unexpected positive microbiological findings, may also indicate certain locations with a high risk of infection/cross-contamination, such as animal transport, lairage, or slaughter hygiene. On-farm food safety procedures play a central role in risk control, which depends on the biosecurity level, including farm infrastructure, feeding methods and water use (well or communal), sanitation measures, pest and pet control, wildlife control, etc. [2]. At harvest level (slaughterhouse), fecal contamination of carcasses and cross-contamination should be avoided through standard hygienic slaughter procedures to reduce the occurrence of both pathogens on meat [17]. In this connection, information on the serological status of a given group of animals upon arrival at the slaughterhouse allows implementation of stricter hygiene measures during slaughter to avoid possible cross-contamination. This is particularly important and specific for *Y. enterocolitica*, which has the highest microbial load in the tonsils rather than in the feces of fattening pigs [18].

In this work, the prevalence of *Salmonella* spp. and *Y. enterocolitica* in pigs from intensive indoor systems (large farms) or extensive small family-owned farm systems was investigated by meat (diaphragm) juice serology and microbiological analyses of the tonsils. The agreement of the results of both methods was compared to discuss the presence/absence of pathogens in seropositive or seronegative animals and the reliability of using the data within the concept of HEIs.

2. Materials and Methods

2.1. Sampling

Sampling of tonsils and diaphragms was carried out using the probabilistic method of simple random selection at slaughter in an abattoir (pigs from large intensive indoor farm

systems) or rural households (pigs from family-owned farms) in the period from September 2021 to March 2022. Ninety-one samples of tonsils and diaphragm meat juice from the same pigs were analyzed, of which 54 samples were from 18 large farms and 37 samples were from 18 small family farms. The average number of pigs fattened on large farms or small family farms was 3793 pigs/year and 8 pigs/year, respectively. According to the data obtained from the authorized veterinary organizations, farms were categorized into three groups based on biosecurity level: 1 (non-compliant holdings), 2 (partially compliant holdings) and 3 (fully compliant holdings). Official farm categorization was based on a questionnaire that included a variety of questions related to current biosecurity measures of each holding including farm infrastructure, sanitation measures, feeding, pest and pet control, etc.

Sampling of the tonsils was performed with a sterile tool by removing them from the set of chest organs, while the root of the diaphragm was taken after splitting the carcass of the same pigs. The samples were stored in sterile bags and transported to the laboratory at 4 °C. The diaphragms were then frozen, and after overnight thawing at refrigerator temperature, obtained meat juice was collected from plastic bags, transferred to plastic cuvettes, and stored at −20 °C until serological analysis. Tonsils were microbiologically tested within 24 h of sampling.

2.2. Microbiological Analysis of Tonsils

For determination of *Salmonella* spp. presence, ten grams of tonsil sample were cut out with scissors, homogenized, pre-enriched in buffered peptone water (Merck, Darmstadt, Germany), and incubated for 18 h ± 2 h at 37 °C ± 1 °C. Selective enrichment was then prepared by adding 0.1 mL of the preincubated culture to 10 mL of Rappaport–Vassiliadis broth (Merck, Darmstadt, Germany) and incubating for 24 h ± 3 h at 41.5 °C ± 1 °C. The obtained culture was then inoculated onto selective agar (xylose–lysine deoxycholate, XLD, Merck, Darmstadt, Germany) and incubated for 24 h ± 3 h at 37 °C ± 1 °C. Suspect characteristic colonies on XLD agar were inoculated on chromogenic IRIS Salmonella® agar (Biokar Diagnostics, Allonne, France). In the case of *Yersinia enterocolitica*, the same amount of tonsils was homogenized in enrichment broth (Peptone, Sorbitol, and Bile salts, PSB, Sigma Aldrich, St. Louis, MO, USA). Then, 1 mL of the original PSB solution in two dilutions (10^{-1} and 10^{-2}) was directly inoculated onto selective agar (Cefsulodin, Irgasan™ and Novobiocin CIN, Merck, Darmstadt, Germany) and incubated for 24 h ± 2 h at 30 °C ± 1 °C. In addition, 10 mL of the original PSB solution was transferred to 90 mL of selective enrichment broth (Irgasan™ Ticarcillin and Potassium Chlorate, ITC, Sigma Aldrich, St. Louis, MO, USA) and both solutions were incubated for 44 h ± 4 h at 25 °C ± 1 °C. Prior to inoculation onto CIN agar, both enriched samples (PSB and ITC) were treated with an alkaline solution (0.5 mL culture + 4.5 mL KOH) for 20 s ± 5 s. Incubation of the inoculated CIN agar was performed for 24 h ± 2 h at 30 °C ± 1 °C. Characteristic colonies on CIN agar (small, round, dark purple center and transparent edge), referred to as "bull's eye", were kept and inoculated for final determination.

2.3. MALDI–TOF Analysis

All retained isolates were sent under controlled conditions to the Ruđer Bošković Institute in the Department of Physical Chemistry for identification by MALDI–TOF mass spectrometry (Matrix-Assisted Laser Desorption Ionization—Time of Flight).

The sample for MALDI–TOF MS analysis was prepared according to the manufacturer's recommendations (Bruker Daltonik, Bremen, Germany). A bacterial colony was spread on a polished steel plate and mixed with 1 µL of 70% formic acid (Fisher Scientific, Madrid, Spain) and dried at room temperature. Each sample was covered with 1 µL of MALDI matrix (a saturated solution of α-cyano-4-hydroxycyanamic acid (HCCA, Bruker Daltonik, Germany)) in 50% acetonitrile and 2.5% trifluoroacetic acid (Sigma-Aldrich, St. Louis, MO, USA) and dried at room temperature. Mass spectra were automatically generated using a microflex™ LT MALDI–TOF mass spectrometer

(Bruker Daltonik, Bremen, Germany) operated in linear positive mode in the mass range of 2000–20,000 Da. The instrument was calibrated with a standard bacterial assay from Bruker. The acquired mass spectra were processed with the computer program MALDI Biotyper 3.0 (Bruker Daltonik, Bremen, Germany) using the default settings. The initial logarithmic value of the result in MALDI Biotyper is in the range of 0–3.0, which represents the probability of correct identification of the isolate calculated by comparing the peaks of the unknown isolate with the reference spectrum in the database. The following identification criteria were used: a result of 2300–3000 indicates a very probable identification at the species level, a result of 2000–2299 indicates a confident identification of the genus with probable species identification, a result of 1700–1999 indicates a probable identification at the genus level, while a result < 1700 is considered unreliable.

2.4. Determination of Salmonella spp. and Yersinia spp. Antibodies in Meat Juice Samples

To obtain the meat juice, the diaphragm samples were placed in bags for homogenization using sterile equipment and thawed for 24 h ± 4 h at 4 °C. Subsequently, 1 mL of meat juice was filled into 1.5 mL plastic cuvettes and refrozen at −20 °C until the start of analysis. The presence of IgG antibodies against *Yersinia* spp. and *Salmonella* spp. was determined using commercial ELISA kits with inactivated antigen (Indical Bioscience GmbH, Leipzig, Germany). Meat juice samples were diluted 1:10 according to the manufacturer's instructions. Each protocol contained 2 positive and 2 negative controls from the ELISA kit. Of the sample, 100 µL was added to the microtiter plate well coated with a specific antigen and incubated at 22 °C for 1 h. After incubation, the wells were washed three times with diluted wash solution (1:10), and 100 µL of the conjugate was added to each well. After incubation (30 min at room temperature) and a second wash, 100 µL of TMB substrate was added to each well, followed by incubation at room temperature without exposure to light. The reaction was stopped after 10 min by adding 100 µL of the stop solution. After the entire procedure, the wells were read using a microplate reader (BioTek ELx800) and analyzed using Gen5™ software (BioTek, Winooski, VT, USA, Version 2.0). The S/P ratio was calculated for each sample, and samples with an S/P ratio $\geq$ 0.3 were considered positive.

2.5. Statistical Analysis

Statistical analysis of the results was performed using the computer program TIBCO Statistica 13.5. The probability of finding the bacterial pathogen in the tonsils of serologically positive compared with serologically negative pigs was determined by measuring the odds ratio (OR) at 95% confidence intervals (CI). Statistically significant differences in (sero)prevalence of *Salmonella* spp. and *Y. enterocolitica* as a function of farm size and biosecurity category were determined using Fisher's exact test at the level of 0.05 (p). The correlation between seroprevalence and prevalence of the bacterial pathogen in the tonsils of the same pig was tested using Cramer's V correlation measure (φc).

3. Results

3.1. Prevalence of Salmonella spp. and Yersinia enterocolitica in Pig Tonsils

The sample was considered positive if the suspect colony on the microbiological medium was confirmed as *Y. enterocolitica* or *Salmonella* spp. by MALDI–TOF mass spectrometry. The farm was considered positive if one of the tonsils tested contained the pathogen, in the case of several samples tested per farm. The results of the (sero)prevalence of both bacteria in relation to the farm size of the pig origin are shown in Table 1. *Y. enterocolitica*-positive pigs from large farms belonged to biosecurity categories 2 (partially compliant) and 3 (fully compliant). From biosecurity category 3 farms, a total of 34 pig tonsils were examined, of which 3 (8.82%) were positive; from biosafety category 2, a total of 15 pig tonsils were examined, of which 2 (13.33%) were positive. No statistically significant difference was found between these two categories ($p > 0.05$).

Table 1. Results of the (sero)prevalence of *Yersinia enterocolitica* and *Salmonella* spp. according to the farm size of the pig origin.

Farm Type	Number of Farms	Number of Samples	N (%) of Positive *Y. enterocolitica*		N (%) of Positive *Salmonella* spp.	
			* S	** M	* S	** M
Large farms	18	54	6 (11.11) [a]	5 (9.26)	26 (48.14) [a]	6 (11.11)
Small farms	18	37	6 (16.21) [b]	1 (2.70)	18 (48.64) [b]	3 (8.11)
Total	36	91	12 (13.18) [c]	6 (6.59)	44 (48.35) [c]	9 (9.89)

* Serologically positive; ** microbiologically positive; [abc] values in the same row marked with the same letter are significantly different at level of 0.05.

The prevalence of *Salmonella* spp. in the tonsils of pigs from large pig farms was 11.11%. In contrast to *Y. enterocolitica*, a statistically significant higher prevalence (26.67%) was found in biosecurity category 2 farms ($p < 0.05$), as only one positive sample (2.94%) came from a biosafety category 3 farm (Table 2).

Table 2. Results of the (sero)prevalence of *Yersinia enterocolitica* and *Salmonella* spp. according to the biosecurity category of farm.

Farm Type	Biosecurity Level	N (%) of Positive *Y. enterocolitica*		N (%) of Positive *Salmonella* spp.	
		* S	** M	S	M
Large farms	1	-	-	3 (11.54)	1 (16.67)
	2	3 (50.00)	2 (40.00)	7 (26.92)	4 (66.66) [a]
	3	3 (50.00)	3 (60.00)	16 (61.54)	1 (16.67) [a]
Small farms	1	-	-	3 (16.67) [b]	1 (33.33)
	2	6 (100.00)	1 (100.00)	15 (83.33) [b]	2 (66.66)
	3	-	-	-	-

* Serologically postive; ** microbiologicallypositive; [ab] values in the same column marked with the same letter are significantly different at level of 0.05.

The prevalence of *Salmonella* spp. and *Y. enterocolitica* in the tonsils of pigs from small farms was lower compared to large farms, but with no statistically significant difference ($p > 0.05$). Most rural households (72.22%) belonged to biosecurity category 2. The prevalence of *Salmonella* spp. was 8.1% (biosafety categories 1 and 2) with no statistically significant difference ($p > 0.05$), while *Y. enterocolitica* was found in only one tonsil sample (2.7%) from a pig originating from biosecurity category 2 farm. Differences in the prevalence of both pathogens within the group of farms as well as between small and large farms were not detected ($p > 0.05$).

3.2. Meat Juice Seroprevalence of Salmonella spp. and Yersinia spp.

According to the serological analysis of the meat juice, the pig is considered seropositive if the S/P ratio of the sample was greater than or equal to 0.3, while the farm is considered positive if at least one sample from the farm was seropositive. Of a total of 91 meat juice samples tested, 13.18% were positive for antibodies to *Yersinia* spp., while 48.35% of pigs had antibodies to *Salmonella* spp. ($p < 0.05$). Most serologically positive pigs against both pathogens were from biosecurity category 2 farms. Summarizing the results by type of farm, a slightly higher seroprevalence of *Yersinia* and *Salmonella* spp. was found in small farms compared to large farms, but with no statistically significant difference ($p > 0.05$). Regarding their biosecurity category, higher seroprevalence ($p < 0.05$) of *Salmonella* spp.

was found in pigs from small farms in biosecurity category 2 compared to pigs from small farms in category 1 (Table 2).

3.3. The Level of Agreement in Detecting Prevalence by Microbiological and Serological Test

Under microbiological examination, *Y. enterocolitica* was isolated from six (6.59%) pigs, of which only one pig (16.67%) also had antibodies, whereas it was not isolated from the tonsils of the remaining 11 (91.67%) pigs with positive serological findings. Accordingly, the association between the two observed variables (φc = 0.027, $p = 0.718$) was low (Table 3). *Salmonella* spp. were isolated from nine (9.89%) pigs, of which six (66.67%) pigs also had antibodies to *Salmonella* spp. No bacteria were isolated from the tonsils of the remaining 38 (86.36%) pigs with positive serological findings. Similarly, the association between the two observed variables was low (φc = 0.121, $p = 0.420$) (Table 4).

Table 3. The correlation between meat juice seroprevalence and prevalence of *Y. enterocolitica* in the tonsils using Cramer's V correlation measure (φc).

	* Negative	* Positive	Total	φc	*p* Value
** negative	74	11	85	0.027	0.718
** positive	5	1	6		
total	79	12	91		

* Serologically; ** microbiologically.

Table 4. The correlation between meat juice seroprevalence and prevalence of *Salmonella* spp. in the tonsils using the Cramer's V correlation measure (φc).

	* Negative	* Positive	Total	φc	*p* Value
** negative	44	38	82	0.121	0.420
** positive	3	6	9		
total	47	44	91		

* Serologically; ** microbiologically.

The association of recovering the bacterial pathogen from the tonsils of serologically positive pigs compared with serologically negative pigs in relation to farm type was shown by the odds ratio (OR) values at 95% confidence intervals (CI), but without statistical significance ($p > 0.05$) in both cases (Table 5).

Table 5. The probability of finding the bacterial pathogen in the tonsils of serologically positive pigs compared with serologically negative pigs indicated by the odds ratio (OR).

Bacterial Species	Farm Type	OR	95% CI	*p* Value
Yersinia enterocolitica	A	2.2	0.203–23.737	0.516
	B	1.56	0.057–42.866	0.791
Salmonella spp.	A	2.36	0.394–14.154	0.346
	B	2.25	0.186–27.224	0.523

A large farm; B small farm.

4. Discussion

4.1. Yersinia (Sero)prevalence

Pigs are usually asymptomatic carriers of the bacterium *Y. enterocolitica* and represent an important potential source of infection for humans, mainly associated with the consumption of raw or undercooked meat [19,20]. The first study on the prevalence of *Y. enterocolitica* in/on the tonsils and mandibular lymph nodes of slaughtered pigs in Croatia was conducted in 2014 [21], reporting the prevalence of 33.33% in tonsils and 10.25%

in mandibular lymph nodes. A recent study [22] conducted in the same slaughterhouses and farm types confirmed the persistence of human pathogenic bioserotype 4/O:3 with prevalence of 43% (95% CI 36.7–49.7) in pig tonsils. Differences in prevalence were found in relation to the type of farm: Integrated farms had a prevalence of 29%, medium farms (collecting piglets from different farms) 52%, and small family farms 40% [22].

In this study, the prevalence of *Y. enterocolitica* in the tonsils of pigs was found to be 6.5%, with 83.33% of the positive samples coming from large pig farms. Most authors associate a higher prevalence with a high production capacity of the farm and the density of the livestock, which was also observed in this work. However, the prevalence observed in our study is lower compared to some European studies and previous reports from Croatia [21–25]. However, the large discrepancy between studies in terms of reported prevalence of *Y. enterocolitica* in pig tonsils at slaughter can be attributed to numerous factors, such as tonsil sampling strategy, slaughter processing (like tying the rectum/removing the head before carcass splitting or not) or the methods used in pathogen isolation and identification [26]. No association was found regarding the presence of *Y. enterocolitica* in the tonsils and biosecurity in the farms, which is opposite to the results of other studies [22,27,28].

The seroprevalence reported in this study was found to be 5% higher than the European average [29], with 27.78% of farms positive. These results are consistent with a similar study by Kiš et al. [13] in Croatia. The large number of *Yersinia*-positive farms was not surprising, considering the seroprevalence of up to 80% found in other studies [3,30]. Seroprevalence data can be very useful in monitoring and planning intervention measures in slaughterhouses and/or can be incorporated into food chain data [13]. In addition, sufficient seroprevalence databases may be used in logistic slaughter decisions, which has proven to be a very useful tool to prevent the spread of *Yersinia* spp. and cross-contamination of pig carcasses during slaughter [31].

The low association of meat juice seroprevalence results and the presence or absence of *Y. enterocolitica* in pig tonsils reported in this study clearly demonstrates the complexity of risk mitigation at both the farm and slaughterhouse levels. This discrepancy may be due to infections that resulted in the production of antibodies after which the microorganisms were removed from the hosts, or it may indicate a recent infection on the farm, during transport, or lairage at the slaughterhouse when a positive microbiological result is found in a serologically negative pig [32]. However, serologic profiling of farms appears to be more reliable tool concerning the opposite results of other studies [32], the high likelihood of missing the pathogen recovery in tonsils due to the background microbiota and the lower sensitivity of cultural methods [33]. An estimation of true prevalence based on microbiological analysis of tonsils may additionally be misinterpreted if poor hygiene practices at slaughter result in cross-contamination [21].

4.2. *Salmonella* (Sero)prevalence

Pigs can be orally infected with *Salmonella* spp. that colonize the tonsils within a very short time (30 min), thus posing a high risk for the introduction of *Salmonella* spp. into the food chain through the processing of pigs at the slaughterhouse [34]. Compared to *Yersinia*, tonsils are of less importance in terms of *Salmonella* contamination of meat at slaughter, even being present in lymphatic tissues of the head region of the animal [34]. European studies have shown that the highest proportion of *Salmonella*-positive samples was observed at the farm level, while the prevalence of *Salmonella* on pig carcasses was much lower [35]. In our study, based on the microbiological analysis of tonsils, the *Salmonella*-positive animals were mostly from farms in the lower biosecurity categories (1 and 2), and the prevalence of 9.8% is consistent with other reports [21,36–38].

The seroprevalence of *Salmonella* spp. in the studied pig population was about 50%, with a slightly higher seroprevalence in small farms, which is consistent with the studies conducted [39,40]. Overall, more than 60% of the sampled farms were seropositive for *Salmonella* spp., which was expected given similar studies [3,41]. Most of the positive

samples were from biosecurity farm 2, indicating an insufficient level and success of biosecurity measures application in these farms.

Large discrepancies between the serologic prevalence of meat juice and the prevalence detected by microbiologic analysis of tonsils have been reported previously. Both cases are possible, i.e., tonsils from serologically positive animals can be negative and vice versa. A study performed even on heavy pigs reported that the association between the actual infection status of pigs and serology was not significant [40,42] and emphasized that the determination of the infection status of pigs at slaughter and the associated risk of the spread of *Salmonella* spp. is only possible by bacteriological examination of different samples and preferably by combining several samples. In a study on inconsistencies between isolation of *Salmonella* spp. from mesenteric lymph nodes and results of serological profiling of pigs at the slaughter line, Nollet et al. [43] found a weak correlation between bacteriological and serological diagnosis of *Salmonella* spp. In a study on the effects of logistic slaughter on the prevalence of *Salmonella* spp. in pigs, the results of [36] showed that post-slaughter contamination of carcasses was partly due to positive herds of pigs previously slaughtered in the same slaughter line, but also to the microbiota present in the slaughterhouse. These findings, as well as our results, show the complexity of interpreting the risk level of herds/farms based on analytical laboratory tools. In this regard, additional analysis of risk factors from farm to slaughterhouse should be included in the final risk management process.

5. Conclusions

Given the high demand, duration, and complexity of performing conventional microbiological laboratory testing for *Y. enterocolitica* and *Salmonella* spp., there are many possibilities and opportunities for using meat juice serology as an indirect method of risk evaluation. The use of meat juice serology can be recommended to categorize farms/herds according to risk, as a basis for determining the order of logistic slaughter, and as a very useful tool to prevent the spread of *Yersinia* spp. and *Salmonella* spp. and cross-contamination of pig carcasses during slaughter. However, serological profiling of farms cannot in itself be a simple solution for decision making by risk managers, if it excludes all other risk factors that may be responsible for the occurrence of natural infection (at farm level) or contamination of pork (at slaughterhouse level).

Author Contributions: Conceptualization, N.Z. and M.K.; methodology, N.Z.; software, M.K.; formal analysis, D.F. and M.K.; investigation, D.F.; writing—original draft preparation, M.K. and D.F.; writing—review and editing, N.Z.; project administration, N.Z.; funding acquisition, N.Z. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the project "Improving professional practice on farm animals and horses at the Faculty of Veterinary Medicine of the University of Zagreb–VETFARM (UP.03.1.1.04.0045) founded by the European Social Fund and the Operational Program Effective Human Resources (2014–2020).

Data Availability Statement: Data are contained within the article.

Acknowledgments: We are grateful to Snježana Kazazić for her assistance in performing MALDI–TOF analysis.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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