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A risk assessment model for *Salmonella* spp. in swine carcasses

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Abstract

Salmonellosis is one of the most important food-borne outbreaks that occurs in the EU/EEA. From the first production stages at slaughter, meat is susceptible to spoilage and can be a substrate for the pathogenic microorganisms growth. Among the pathogens, the presence of *Salmonella* is mainly due to mishandling during the evisceration stage. For the year 2019, according to the collected data from MSs, on the 17.9% of all food-borne outbreaks, the presence *Salmonella* was confirmed. Pork meat is considered as one of the four most commonly reported foods in cases of salmonellosis. For the training purposes of this project, *Salmonella* isolation and identification along with RA for carcass contamination, was performed. Pig carcasses were sampled using the non-destructive technique. The sampling took place post dressing and before the stage of chilling. For the *Salmonella* detection, a three phases process was performed (pre-enrichment, enrichment, isolation). A total of 757 samples were collected, 19 were found to be positive for *Salmonella*. The most common was found to be *Salmonella* Derby, which was identified eight times. The main objective of the project was to determine the prevalence of *Salmonella* spp. in swine carcasses. Moreover, certain parameters were evaluated in terms of their influence on the prevalence of *Salmonella*. A stochastic simulation model was developed in Microsoft Office Excel 2019 by using the add-in @Risk v.8.1. The prevalence was estimated to be 2.6%. For the pigs sampled, the average value of the distance from farm to slaughterhouse was 200.92 km. Additionally, the average weight of the carcasses was 127.97 kg. The prevalence of *Salmonella* between the samples that came from farms with a distance above the average, was higher by 1.7 units, while the prevalence for the samples with weight above the average was higher by 0.2 units. According to the stochastic model, it is specified that the prevalence is higher with greater distance, and there is an 8.1% probability the prevalence will exceed the legislation's – hygiene criteria. In addition, the prevalence of *Salmonella* was shown to increase, as well in the case of samples from weightier animals, but to a lesser extent.

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1. Introduction

1.1. Food safety and hygiene

During the slaughter of animals and meat processing, the possibility of contamination by mishandling is high. From the first stages of the slaughter, meat is susceptible to spoilage and can be an ideal substrate for the growth and multiplication of pathogenic microorganisms. The application of inappropriate hygiene practices during handling, processing, storage, and distribution, catalyses the type and extent of contamination, causing unwanted degradation of quality and potentially serious consequences for the safety and health of consumers. During the last years, continuous improvement actions are carried out in the production processes with the main aim of implementing suitable food safety protocols. Despite these, incidents and their graveness remain high, causing increased concern and a considerable rise in uncertainty. In cases involving the growth of pathogenic microorganisms in meat, the type or intensity of the infection may be influenced by factors such as the origin or other specific characteristics of the animal (Koutsoumanis and Sofos, 2004). Among the main causes of contamination in meat are, direct contact with faeces residues, hides during the skinning process, lymph nodes and gastrointestinal tract during the process of evisceration (Mann et al., 2016; Mrdovic et al., 2017; Peruzi et al., 2021).

According to EU Regulation 853/2004, 7°C is defined as the maximum storage temperature for carcasses, which needs to be applied once the dressing stage is been completed. At this temperature, most of the pathogenic microorganisms do not grow, but the activity of some of them is not completely inhibited. Among the pathogens is the presence of *Salmonella*, which is mainly due to mishandling during the evisceration stage (Choi et al., 2013; Sánchez-Rodríguez et al., 2018, Grispoli et al., 2020).

1.2. Reported food-borne data

Salmonellosis is one of the most significant food-borne outbreaks that occurred in the EU/EEA on an annual basis. It is the second most frequent gastrointestinal infection in the human population. First in the ranking is campylobacteriosis. Between the years 2018 and 2019, the reported cases of salmonellosis in the European Union (EU) remained at the same level, interpreted as 20 recorded cases for every 100,000 people. During the last 5 years, there have been stabilising trends regarding the cases of *Salmonella* Enteritidis, as well. In absolute numbers, the confirmed cases, for 2019, reached the level of 88,000. From the collective data of the 23 Member States (MS), for 2019, a total of 926 salmonellosis cases were recorded. More than 9,000 people fell ill, leading to seven declared deaths. For the 17.9% of all food-borne outbursts, *Salmonella* was confirmed as the main cause, while 72.4% of them were due to *S. Enteritidis* (EFSA, 2021).

Pork meat and its products are one of the four most commonly reported foods in cases of salmonellosis. For the years 2017 to 2019, the officially declared data, of the results of the *Salmonella* control by the food companies, show continuously low percentages of positive samples in pork carcasses. Nevertheless, the presented values, are often lower than the results of the inspections carried out by the competent authorities. From the MS' reports on the serotyped isolates, derived both from food and animal sources, 12% concerned the case of pork origin. The serotyped isolates with the highest incidence responsible for infections in the human body were *S. Infantis* at 29.7%. Followed by *S. Enteritidis*, the single-phase variant of *S. Typhimurium*, *S. Typhimurium* and finally *S. Derby*, with percentages of 6.9%, 4.5%, 3.9% and 3.7%, respectively (EFSA, 2021).

1.3. Legislation's requirements

According to Annex I of the EU Regulation 2073/2005, it is stated that after three successive positive samples, but no longer after five positives, the food business operators must adopt immediately suitable preventive measures. For instance, the general upgrading of hygienic conditions during slaughter or revaluation of the process controls. Therefore, it must be certified that the percentage of carcasses that do not fulfil the process hygiene criteria, is less than 6%.

The EU Regulation 218/2014, inserts further information regarding the official inspection controls concerning porcine species, with the aspect of respecting the application of the microbiological criteria. At the slaughterhouse, the business operator should carry out a fixed annual sampling plan with a minimum of 49 samples. Each sample from a different animal. The competent authority must plan the method most suitable for each slaughterhouse.

In the case of having one or more positive samples, out of the total 49 animals tested, a $\geq 6\%$ *Salmonella* prevalence is assumed, with a 95% confidence margin. As a result, the competent Authority shall investigate further the possible non-conformities to determine if the critical percentage of 6% is exceeded or not. This percentage can also be interpreted as the minimum percentage of samples that need to meet the legislation – hygiene criteria in the official sampling plan before corrective actions are needed.

Furthermore, the sampling plan can be modified accordingly and comprise smaller number of samples. This can be achieved only with relevant approval by the official veterinarian authority, after taking into account the risk assessment of the slaughterhouse, and aspects such as the size of the slaughterhouse, hygienic conditions, the facilities of the establishment, number of suppliers, distance between suppliers' farms and premises. The implementation of all samplings should always be in respect of EU Regulation 2073/2005.

All results and data from the business operator's self-control and from authorities sampling, must be reported in an annual base to European Commission in accordance with 2003/99.

2. Description of work programme

2.1. Importance

Active enrolment in the *Salmonella* isolation and identification along with RA for carcass contamination, will cover all aspects of the risk assessment process, including knowledge transfer to state veterinarians and food business operators. The topic of choice is of extreme actuality and arises from the background of a progressive streamlining of food controls on abattoir on behalf of visual inspection and limitation of palpation and cuts. For a comprehensive risk assessment, this approach has to consider the presence of bacteria on the carcass as per regulation 2073/2005.

2.2. Activities/Methods

This study was carried out at a slaughterhouse in central Italy between October 2018 and October 2021. During this period, 757 pig carcasses were sampled using the non-destructive technique. The four sampling sites tested for *Salmonella* presence on swine carcasses were: rump, belly, thorax, and neck. The sampling areas of each of the four sites were approximately 100 cm², like a square with 10 cm length on each side. The sampling took place post-dressing and before the stage of chilling.

The technique used is the following: for the sampling, a sponge in a sterile bag (Hydrated Spec-Sponge[®] Bags) is moistened in 10 mL of peptone water. Make sure that the sponge is adequately soaked. After identifying the sampling sites, delimit the four areas each of 100 cm². Apply on both halves of the carcass, by exerting sufficient pressure to the skin surface area. Sterile disposable gloves should be used, to avoid any contamination of the samples. The sponge should be swiped across the surface to be sampled horizontally, vertically, and diagonally, approximately 10 times in each direction, inside the limits of the designated areas. Then return the sponge into the sterile bag, seal it and label it with lot number of the sampled carcass.

Salmonella detection involves a process of three successive phases. The first is pre-enrichment, the second is enrichment, and the last one the isolation. For the pre-enrichment phase, test tubes containing 25 mL of peptone water were used (ISO 17604/2015). These tubes were poured inside the sterile sponge bags and after sampling they were incubated at 37°C for 24 h. For the enrichment phase, 0.1 mL was taken from the liquid derived from the sponge, after pre-enrichment, and sown in tubes containing a selective broth for the isolation of salmonellae, Rapport-Vassiliadis (ISO 6579-1 2017/AMD 1:2020). These tubes were incubated at 42°C for 24 h. This broth was developed specifically to exploit the four characteristics that differentiate *Salmonella* from other Enterobacteriaceae, namely: endurance to high osmotic pressures, growth at lower pH values, malachite green resistance, minor nutritional requirements. For the isolation, the samples were sown, by using sterile loops, on Petri dishes containing xylose lysine deoxycholate (XLD), a selective medium for the isolation of salmonellae, suitable for clinical and food samples. These plates were then incubated at 37°C for an additional 24 h. Salmonellae use xylose and decarboxylate lysine, thereby changing the pH to higher alkalinity levels. Change of the soil's colour is observed. The initial light red turns to bright red/violet. Furthermore, salmonellae presence can be spotted from the black-coloured colonies. The hydrogen sulfide, which is produced as a catabolite, forms a bind with the ammonium iron citrate, found in the soil, precipitating in the form of a black compound (ISO 6579-1 2017/AMD 1:2020).

Colonies showing phenotypic characteristics attributable to the genus *Salmonella* were isolated, cultivated in purity, and stored in a freezer at -80°C for subsequent analysis. The stems were then thawed and grown in brain–heart infusion broth at 37°C for 24 h. Subsequently, a suspension in physiological solution of the second degree of the McFarland scale was prepared for each bacterial stem. This suspension was inoculated in an API 20E diagnostic gallery and incubated at 37°C for 24 h. API 20E is a standardised system used, not only for the identification of Enterobacteriaceae but for other non-demanding Gram-negative bacilli as well. It includes 21 miniaturised biochemical tests, in addition to a specific database. The API 20E gallery consists of 20 microtubes, containing dehydrated substrates. The tubes are inoculated with a bacterial suspension that reconstitutes the media. The reactions produced during the incubation period result in spontaneous colour changes or may be revealed by the addition of reagents. The reading of these reactions is carried out using the reading table while the identification is obtained with the analytical index or with the identification software. The stems identified as *Salmonella* spp. were sent to the 'Togo Rosati' Experimental Zooprophyllactic Institute of Umbria and Marche for serotyping (ISO 6579-1 2017/AMD 1:2020).

Serological typing is carried out through serum agglutination, with a rapid method on a slide. This method is applied to the identification of somatic O antigens and H flagellar antigens and is based on the identification scheme developed by Kauffmann-White, Le Minor (Grimont and Weill, 2007).

For the 19 positive samples, the serotype was specified, as described above. From more to less frequently identified: *Salmonella derby*, eight times; *Salmonella typhimurium* and *Salmonella London*, three times; *Salmonella give* and *Salmonella Brandenburg*, two times; *Salmonella gold coast* one time respectively (Figure 1, Table 1).

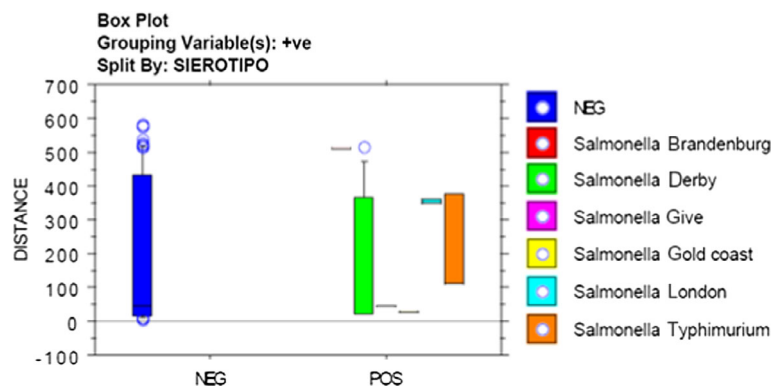


Figure 1: Box Plot – Serotype grouping with distance

2.3. Application of simulation model

To analyse the risk and calculate the prevalence, a stochastic simulation model was developed in Microsoft Office Excel 2019 with the use of the add-in programme @Risk v.8.1 for Excel (Palisade, Ithaca, USA). @Risk is based on a Monte Carlo simulation that can provide beneficial outcomes and allow to overcome uncertainty in quantitative analysis. Monte Carlo simulation can perform risk analysis through the substitution of individual points of uncertain inputs with the distribution of possibilities. These are randomly tested over and over, for many interactions, and the model calculation to create large sets of possible data which can then be further analysed.

A total of 757 samples were collected and the data were processed with the application of @Risk. Of that 19 samples were found to be positive for *Salmonella*. The prevalence of *Salmonella* was estimated to be 2.6%. The pigs came from farms in various parts of Italy (Figure 2). The distance from farm to slaughterhouse ranged from 7.6 to 584 km and the average value was 200.92 km (Figure A.1a). The weight of the carcasses ranged from 51 up to 207.8 kg with an average of 127.97 kg (Figure A.1b). The prevalence between the samples that came from farms with a distance greater than 200 km (distance above the average), concerning the prevalence of *Salmonella* for all the samples, was higher by 1.7 units, with an estimated prevalence value of 4.3%. The stochastic model gives a direct correlation between the distance and the expected prevalence of *Salmonella*. A possible cause, can be the increased length of stay, of the animals, in the transport vehicles with inadequate hygiene conditions and close contact among animals (Simons et al., 2016). The considerable higher prevalence value (4.3%) designates the need to increase monitoring and sampling frequency in these

cases (Figure A.2). On the other hand, the prevalence of *Salmonella* for the samples that weighed more than 130 kg (weight above the average), concerning the prevalence of *Salmonella* for all the samples, was higher by 0.2 units, with an estimated prevalence value of 2.8% (Figure A.3) (Graphs Appendix A).



Figure 2: Locations of the farms (a) Northern Italy and (b) Central Italy – Region of Umbria (Google Maps). Yellow pins: Farms with samples tested positive for *Salmonella*

2.4. Additional relevant activities and learning opportunities

Besides the specific activities on the risk assessment model for *Salmonella* spp. in swine carcasses, the fellow participated in a full range of activities of the research unit, which is indeed a group young and committed, so the fellow took part in the exciting and numerous activities of the unit: participations to the master degree workshops and the continuing education training for the public health and risk assessment. Moreover, the fellow has fully involved in the organisation and participation in the monthly scientific events of the master degree in Veterinary public health and food hygiene (Appendix B).

3. Conclusions

The main objective of the project was to determine the prevalence of *Salmonella* in swine carcasses from regular and emergency slaughter. This research will clarify the role of slaughterhouses and procedures as a source of pathogens' contaminations and focus the attention on the importance of RA along with the accurate and detailed inspection of the carcasses despite modern trends and revisions of procedures. For all carcasses sampled, additional accompanying data were collected for processing and analysis. All information came from the competent audit authority responsible for the operation of the sampling slaughterhouse. Furthermore, certain parameters were evaluated in terms of their influence on the prevalence of *Salmonella*. According to the stochastic model, it is specified that the prevalence is higher with greater distance, and there is an 8.1% probability the prevalence will exceed the critical percentage of 6%. In addition, the prevalence of *Salmonella* shown to increase, as well in the case of samples from weightier animals, but to a lesser extent. The probability the prevalence will exceed the critical percentage of 6%, is 0.1%.

Risk assessments are beneficial studies that in fact can help decision-making. Based on the RA results, FBOs can achieve more targeted resource management. The qualitative model's outcome can be utilised for the reassessment of existing priorities in the inspection technics. Moreover, interventions with great efficacy are made easier to plan and apply throughout the production chain (Pouillot et al., 2012, Costa et al., 2020).

Table 1: Data of samples tested positive in *Salmonella*, including the identified serotype, the lot number – slaughter number, the category, the farm code, Distance farm to slaughterhouse, weight of carcasses, breeder name, the freezer number of the sample when archived

	Slaughter number	Category	Farm code	Distance in km	Weight in kg	Breeder name	Internal reference number
Serotype							
<i>Salmonella</i> Give	043177	S	020TR***	46.6	132.6	B*****	977
	043176	S	020TR***	46.6	121.4	B*****	976
<i>Salmonella</i> Gold Coast	113275	S	039PG***	25.9	144.4	L*****	979
<i>Salmonella</i> Derby	120278	S	163CN***	518	86.2	D*****	980
	120304	S	027PG***	21.5	128	B*****	982
	120313	S	027PG***	21.5	137	B*****	983
	120302	S	006PG***	22.3	131.8	U*****	981
	190236	S	094VR***	365	121	S*****	995
	190237	S	094VR***	365	117.8	S*****	996
	190238	S	094VR***	365	129	S*****	997
	190239	S	094VR***	365	139	S*****	998
<i>Salmonella</i> Brandenburg	127259	S	019CN***	511	138.4	V*****	984
	134234	S	019CN***	511	118.4	V*****	985
<i>Salmonella</i> Typhimurium	148272	S	107CR***	377	156.2	F*****	988
	148273	S	107CR***	377	163.6	F*****	989
	176199	S	006PG***	22.3	121.4	U*****	994
<i>Salmonella</i> London	351184	S	027VR***	351	133.8	A*****	1000
	351188	S	027VR***	351	132.8	A*****	1001
	351283	S	094VR***	365	111.8	A*****	1002

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Abbreviations

AGES	Austrian Agency for Health and Food Safety
BfR	Bundesinstitut für Risikobewertung
EEA	European Economic Area
EFET	Hellenic Food Authority
EU-FORA	European Food Risk Assessment Programme
FBO	food business operator
ISO	International Organization for Standardization
MS	Member State
RA	risk assessment
UniPG	University of Perugia
XLD	xylose lysine deoxycholate

Appendix A – Distribution Graphs @Risk

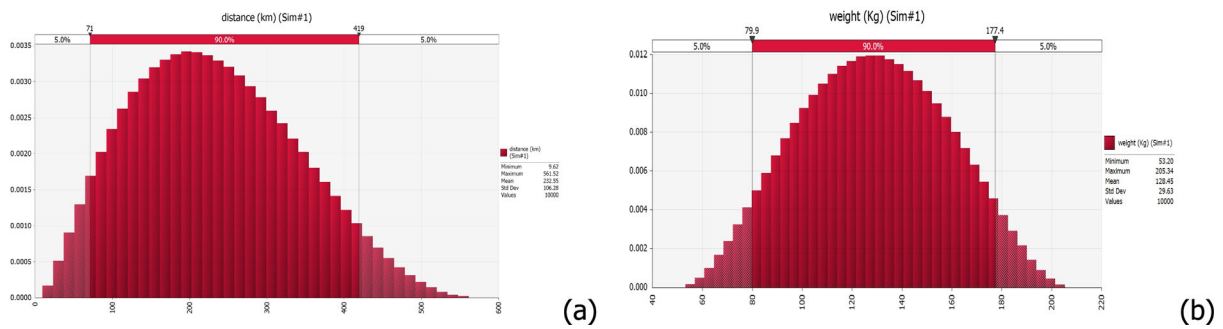


Figure A.1: (a) Distribution of distance values (farm to slaughterhouse), (b) Distribution of weight values

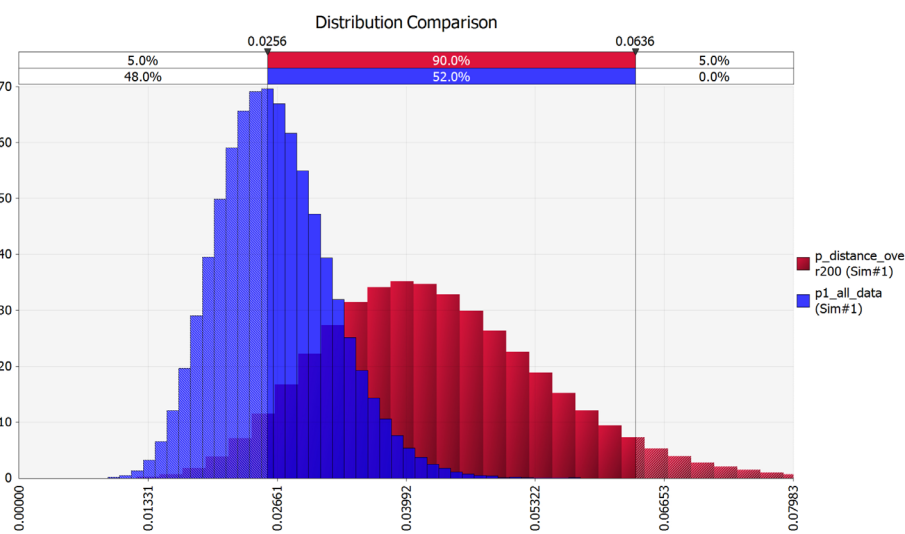


Figure A.2: Prevalence of *Salmonella* in all samples (blue), in comparison with prevalence of *Salmonella* for the samples from farms with distance 200 km and greater (red)

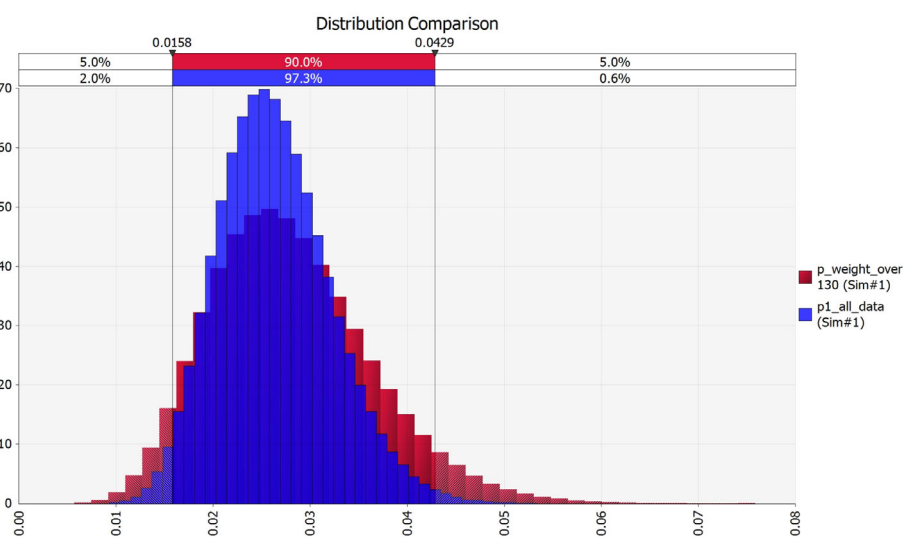


Figure A.3: Prevalence of *Salmonella* in all samples (blue), in comparison with prevalence of *Salmonella* for the samples with weight of 130 kg and up (red)

Appendix B – Side projects and activities throughout the fellowship

Alongside of the main risk assessment project, the fellow had participated actively in all the ongoing projects of the department:

- 1) eLearning courses on Quantitative risk analysis models in Excel, with @RISK, Palisade.
- 2) Participation in the preparation and writing of research papers and ongoing projects of the department: A review manuscript on *E.coli*, currently untitled project/A quantitative risk assessment of *Listeria monocytogenes*/A study on the application of natural extracts as alternatives to sodium nitrite in canned meat/Hygienic Characteristics and Detection of Antibiotic Resistance Genes in Crickets (*acheta domesticus*) Breed for Flour Production/ Evolution and Antimicrobial Resistance of *Enterococci* Isolated from Pecorino and Goat Cheese.
- 3) Regular visits to a slaughterhouse in Umbria Central Italy, for the observation of animal's behavior prior to and after certain animal-welfare interventions.
- 4) Participation in the experimental part of undergraduate student's thesis project: Effect of packaging and storage conditions on some qualitative characteristics of beef meat.
- 5) Webinars on Programming with Python: Getting started with Python/Python Data Structure
- 6) Presentation of the Eu-Fora fellowship programme to Erasmus and undergraduate students of UniPG.
- 7) Day trip to a meat processing company in Umbria Central Italy, for the supervision of the production line, processing and packaging of Chianina beef meat.
- 8) Day trip to an automatic – robotic milk farm in Umbria Central Italy, where cattle moving and handling techniques were examined on Holstein Friesian cattle, in accordance with animal welfare principals.
- 9) Cibo Sovrano. Le guerre alimentari globali al tempo del virus – "Sovereign Food. Global food wars at the time of the virus" by Maurizio Martina, Vice Director of the Food and Agriculture Organization (FAO), Round table discussion.
- 10) EU-FORA Training Courses:
 - Induction training of the European Food Risk Assessment Fellowship Programme (EFSA) (11–29 January 2021).
 - Module 1 training of the European Food Risk Assessment Fellowship Programme – Risk Communication (BfR–EFSA) (22–26 March 2021).
 - Module 2 training of the European Food Risk Assessment Fellowship Programme – Emerging Risks (EFET–EFSA) (7–14 June 2021).
 - Module 3 training of the European Food Risk Assessment Fellowship Programme – Data Collection and Reporting (EFSA) (4–7 October 2021).
 - Module 4 training of the European Food Risk Assessment Fellowship Programme – Novel Foods (AGES–EFSA) (22–26 November 2021).