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# Effect of uranium on the radiosensitivity of Salmonella spp. in pork meat

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## Abstract

*The presence of natural radionuclides in the chain of pig meat production, especially in food and feed additives (premixes), as the most important mutagenic factor, could significantly affect the variability of Salmonella isolates in the pork chain. In the first stage of the study, outlined in this paper, the goal was to determine if radioactivity has any effect on survival/growth of Salmonella in pigs meat.We studied six strains of Salmonella that were exposed to four levels of radioactivity.*

*The differences in Salmonella counts between pork meat homogenates with or without added radioactive uranium were following: the Salmonella counts ($log_{10}$ CFU/ml) in control meat homogenate (without added radioactivity) increased from the level of around 7.4 logs (at 30 min storage) to around 9.0 log*s*, due to growth of the pathogen. No significant differences in this general pattern were observed between the 6 Salmonella serovars tested in the control meat homogenates (without added radioactivity). On the other hand, Salmonella counts in meat homogenate with added radioactivity $^{238}U$ (1, 2, 5, 10 kBq) decreased (by around 1-1.5 logs) practically immediately after addition of the uranium, compared with control meat homogenates. Subsequently, Salmonella also grew during storage of meat homogenate with added radioactivity, but to a lesser extent than in control meat homogenates. Salmonella enteritidis (2) had the highest factor reduction in meat homogenates with radioactivity added $^{238}U$ (1, 2 kBq). It had the highest reduction factor around 1.57 to 1.67 logs. In contrast, Salmonella tiphimurium (3) had the highest factor reduction in meat homogenates with radioactivity added $^{238}U$ (5, 10 kBq), which ranged from 1.83 to 1.96 logs.*

**Key words: Salmonella spp., meat, natural radionuclide - uranium, radiation, radio sensitivity**

## 1. Introduction

Bacteria from Enterobacteriaceae family, *Salmonella spp.*, are the most common contaminants in animal products. Only very few *Salmonella* serovars of over 2000, actually cause the vast majority of human foodborne salmonellosis cases. At the global level, *S. Enteritidis* and *S. Typhimurium*, together, have been the most common (77.1-78.3%) cause of outbreaks of foodborne salmonellosis in the period 1993-1998, and the ratio between the two serovars was 3:1 in favour of *S. Enteritidis* [1]. It is important to note that the source of infection in most outbreaks caused by *S. Enteritidis* were eggs and egg products. On the contrary, the source of infection in most outbreaks caused by *S. Typhimurium* was mostly pork, and to a lesser extent beef. They were followed by serovars *S.* Agona, *S.* Virchow, *S.* Newport, *S.* Brandenburg, *S.* Derby and *S.* Braendrup. In 2003, *S.* Virchow was the third most common serovar isolated in human cases.

Main natural reservoirs of *Salmonella* are swine [2,3] and poultry [4], than rodents, ruminants and carnivores. Many other species are infected, thus they are widely spread.

The sources of human infection are farm domestic animals, and other animals that live in domesticated areas as pests (mice and rats). Humans are rarely infection source, but still have an important part in *Salmonella* spreading, since they can contaminate the food through their feces. *Salmonella* could be transmitted by contact from human to human, especially in case of persons who have deficient immunity (liver cirrhosis, leukemia, lymphoma, HIV). In the last years there have been reports of *Salmonella epidemics* in children and neonatal wards, in regard to means of spreading and durance epidemic was transmitted by objects and contact. In most European countries, gastroenteritis is caused by *S. enteritidis* and *S. typhimurium* [5]. *Salmonella spp*. is considered firstly pathogenic for animals, and to lesser extent for humans, for human salmonella born diseases there should be a higher infectious dose of agents, measuring for some types of salmonella more than million, even billion germs. Such high amount of *Salmonella* could only be found in food originating from infected animals (in which salmonella was spread during life or post mortem) or food subject to secondary contamination by humans. This is the reason why salmonellas are the most common cause of food poisoning [6].

Today, mainly for pragmatic reasons and based on cautionary principle, all *Salmonella* strains are treated as having a potential to cause human foodborne salmonellosis. However, it is known that the strains commonly present in animal feed often serotypically and/or phenotypically differ from the strains commonly present in pigs and/or pig meat. Some advantageous phenotypic variations (e.g. better survival and/or growth ability) can contribute to the natural selection of certain *Salmonella* strains along the food chain and, ultimately, their ability to reach the consumer. One of the factors that could potentially affect the strain diversity, and thus the selection process, is the mutagenic potential of natural radioactivity present at key points of the meat chain (e.g. farm, animals, meat). In Serbia, data on the diversity of *Salmonella* isolates in the pork meat, and their potential relationship to natural radioactivity, are very scarce or lacking.

Pork is a major source of human salmonellosis [7, 8, 9] and source of approximately 20% / 5-30% / total cases in humans [10]. However, reducing the risk of Salmonella, through pork meat, can significantly contribute to the protection of human health.

Uranium and its descendants are significant alpha emitters, but due to the short range of alpha particles, harmful radiation effect is manifested only when entering into a living organism and causing specific intoxication effect that depends on the way of introduction (ingestion, inhalation). Alpha particles penetrate through the tissues of the body, causing ionization effect, and then leading to the formation of free radicals resulting in the changes in molecules, that are very essential for the functioning of cells. Since natural radionuclides are mainly alpha emitters in which the specific power of ionization and hence the relative biological effectiveness (RBE) for living organisms is very high (RBE is higher by a factor of 20 compared to gamma and X rays), the effects on living organisms even at low concentrations of natural radionuclides in the environment can be significant.

In the use of fertilizers and supplements for animal feed commonly used in Serbia (mono and dicalcium phosphate), the content of uranium 238 in the soil increased, as well as in animal feed and all this resulted in the increase of uranium in plants and other links in the food chain, such as pork meat. The pork chain, considering the share of pig meat in the total meat chain, is one of the important routes of contamination of people by natural radionuclides. In addition, the presence of natural radionuclides in the chain of pig meat production, especially in food and feed additives (premixes), as the most important mutagenic factor could significantly affect the variability of isolates of *Salmonella* in the pork chain. This problem has become particularly acute in recent years when, in the industrial production of pig and poultry feed, natural phosphates started to be used as a component, where the content of natural radionuclides can reach very high values [11, 12]. Information about potential effects of natural ionizing radiation on health of the general population, health effects of high and lower doses and radio-ecological areals, have been presented for Serbia [13]. The distribution of uranium in the organism of animals is also important [14].

To avoid the toxicity of radionuclides there are ionizing irradiation methods for elimination of bacteria in plant and animal products. In order to eliminate the *Salmonella spp*. irradiation is used, that unlike our case is not natural but of artificial origin, which represents one option in successful fight against the pathogenic *Salmonella*, saving the sensor characteristics of meat products. To reduce or eliminate viable *Salmonella* spp. cells in meat, various doses of ionizing radiation have been suggested in the published literature [15-22]. The differences are due to different initial contamination and sensitivity variability of *Salmonella spp.* used in different studies. Eliminating *Salmonella spp*. from meat using ionizing radiation, according to international organizations (WHO, FAO, IAEA), is one of the most efficient *Salmollella spp.* control methods, which also enables the maintenance of desirable sensory properties in the treated foods. Based on extensive experimental data, a recommendation has been given by the Commission of Experts WHO, FAO and IAEA to use ionizing radiation in doses up to 10 kGy for the purpose of decontamination of poultry meat, pork and beef [23].

For the reasons indicated above, the main aim of our long-term investigations is to determine whether and in which way natural radionuclide - uranium in food and feed additives (premixes), in the meat chain, affects microbial meat safety. In the first stage of the study, outlined in this paper, the goal was to determine if radioactivity has any effect on survival/growth of *Salmonella* in pork meat. So, knowing that radioactivity affects the reduction in growth of salmonella, then objective of the present study was to determine their relationship in case of different uranium levels.

## 2. Material and Methods

Six Salmonella strains were used in the study: Salmonella enterica subsp. *enterica* serovar *Typhimurium* (ATCC 14028) (1), *Salmonella enterica* subsp. *enterica* serovar *Typhimurium*–(isolates from feed for pigs) (2), *Salmonella enterica* subsp. *enterica* serovar *Typhimurium* – (isolate from pig organs) (3), *Salmonella enterica* subsp. *enterica* serovar *Enteritidis* (ATCC 13076) (1), *Salmonella enterica* subsp. *enterica* serovar *Enteritidis* (isolates from feed for pigs) (2), *Salmonella enterica* subsp. *enterica* serovar *Enteritidis* – (isolate from pig organs) (3). Each strain was grown in Brain Heart Infusion broth (Becton, Dichinson and Co.) for 48 hours at 37$^{o}$C, and then sub-cultured in fresh broth (24 hours at 37$^{o}$C). The cell count in the final broth culture was determined by using a densitometer (DEN-1, Grant-bio, UK), and then diluted in appropriate volume of sterile saline so to achieve $10^6$ CFU per ml in this, the primary *Salmonella* suspension (PSS). Then, 10 ml of the PSS was mixed with 10 g of sterile pig meat (Slaughterhouse "ZZ Trlić") and 80 ml of sterile saline, so to obtain the inoculated pig meat homogenate (IAFH). The IAFH was then divided into two portions: one portion had an equal volume of a solution of uranium of (1 kBq, 2 kBq, 5 kBq and 10 kBq) radioactivity added (IAFH+U; "treatment"), while the other portion had an equal volume of sterile saline added (IAFH; "control" without uranium). Both IAFH+U and IAFH were stored at 8-10$^{o}$C, and each was sampled periodically to determine *Salmonella* counts, after 30 min, 24 hours and after 2 days, by standard spread-plating methodology using Plate Count Agar (HIMEDIA).

Natural radioactivity is expressed in the form of uranyl nitrate hexahidrata. We took the next dilution of crystals:

U-400 mg/l-(0.4 mg/ml)-(0,1688g of uranyl nitrate to 200 ml/$UO_2$ $(NO_3)$ 2 x $6H_20$

U-800 mg/l-(0.8 mg/ml)-(0,0844g of uranyl nitrate to 50ml/$UO_2$ $(NO_3)$ 2 x $6H_20$

U-2000 mg/l-(2 mg/ml)-(0,2109g of uranyl nitrate to 50ml/$UO_2$ $(NO_3)$ 2 x $6H_20$

U-4000mg/l-(4 mg / ml)-(0,4219g of uranyl nitrate to 50ml / $UO_2$ $(NO_3)$ 2 x $6H_20$

This dilution of appropriate concentrations of 1000Bq / 2000Bq / 5000Bq / and 10000Bq. They are made at the Institute for Technology of Nuclear and Other Mineral Raw.

Why was this dose of uranium chosen? Practice has shown, especially for this case, that imported premixes had a dose of 1 kBq to 5 kBq. Therefore, it was decided to use the dose of 10 kBq in the case as an example of emergency situation. So we get all the possible optional doses that would affect the behavior of *Salmonella* strains.

## 3. Results and discussion

The differences in *Salmonella* counts between pig meat homogenates with or without added radioactive uranium (1 kBq) are shown in Figure 1. The *Salmonella* counts ($log_{10}$ CFU/ml) in control pig meat homogenate (without added radioactivity) increased from the level of around 7.4 logs (at 30 min storage) to around 9.0 logs, due to growth of the pathogen. No significant differences in this general pattern were observed between the six *Salmonella* serovars tested in the control feed homogenates (without added radioactivity).

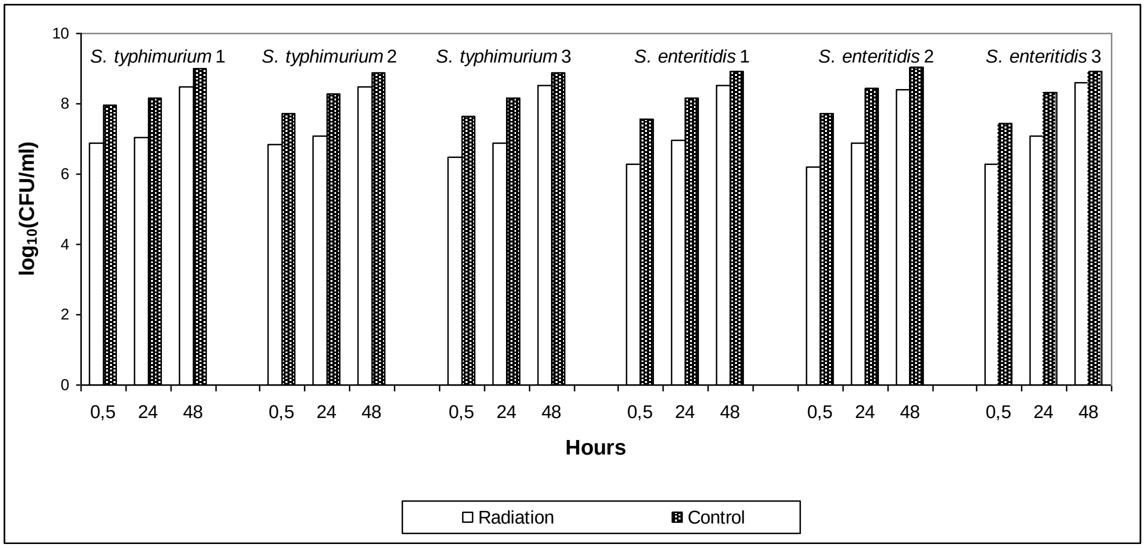


**Figure 1.** ***Salmonella*** **counts in inoculated meat homogenate without uranium (control IAFH) and with uranium of 1 kBq radioactivity (IAFH+U) during storage**

On the other hand, *Salmonella* counts in meat homogenate with added radioactivity (uranium 1 kBq) decreased (by around 0.9-1.5 logs) practically immediately after addition of the uranium, compared with control feed homogenates. Subsequently, *Salmonella* also grew during storage of the feed homogenate with added radioactivity (especially on the second day), but to a lesser extent than in control feed homogenates. The ultimate *Salmonella* counts reached in the presence of added radioactivity were significantly reduced compared with those in the control homogenate: by around 0.5-1.3 logs *S. Typhimurium strains*, or around 0.3-1.6 logs *S. Enteritidis* strains. During the period from 30 minutes to 24 hours, the reduction factor remained at the same level. Only in S. Typhimurium (2) reduction factor increased from 0.87 logs to 1.20 logs. Furthermore, as indicated above, the observed added radioactivity-induced reduction rates were more prominent in *S. Enteritidis* then in *S. Typhimurium*, especially on the first day. What is important for us this particularly applies to *S. Enteritidis*-isolates from animal feed (2) reduction factor was the highest in all three phases of measurement. After the first measurement it was 1.54 logs, after 24 hours 1.57 logs and after 48 hours it amounted to 0.63 logs. In comparison to other S*almonella* strains, the highest factor in reducing the radioactivity and the smallest growth were observed when radioactivity was added.

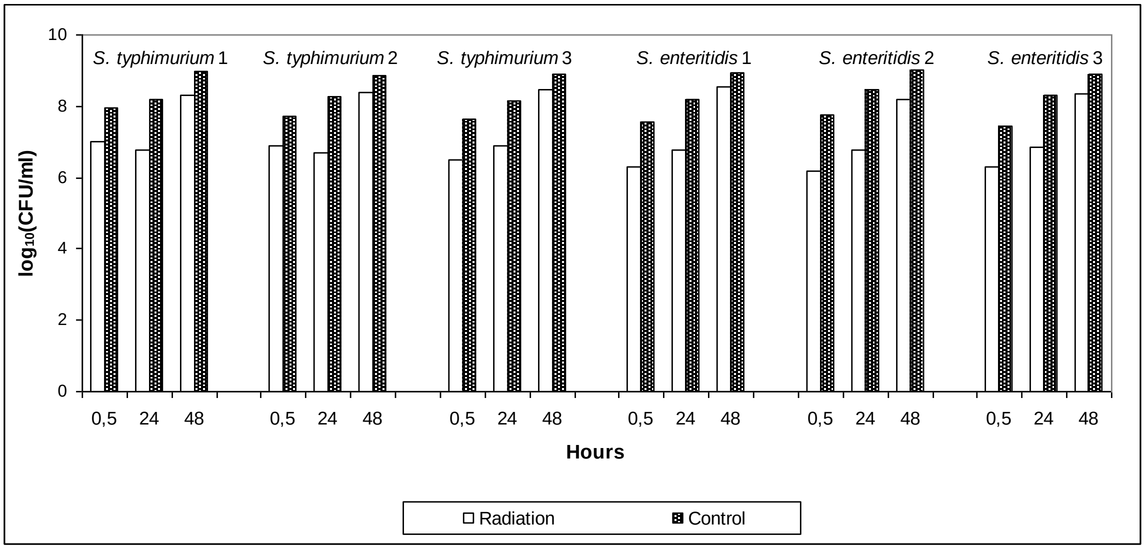


**Figure 2.** ***Salmonella*** **counts in inoculated meat homogenate without uranium (control IAFH) and with uranium of 2 kBq radioactivity (IAFH+U) during storage**

The *Salmonella* counts ($log_{10}$ CFU/ml) in pig meat homogenate with added radioactivity (uranium 2 kBq) were similar to that of the group with the added activity of 1 kBq. The differences in *Salmonella* counts are shown in Figure 2. The greatest reduction was after first day: by around 1.2-1.7 logs and the lowest on the second day: by around 0.4-0.7 logs. Reduction factor increased from the starting control at 30 minutes to 24 hours in all strains, and is higher than the groups where 1 kBq of radioactivity was added. It ranged from 0.09 logs in S. Typhimurium (3) to 0.76 logs in S. Typhimurium (2).

The same as in the previous group, the highest factor of reduction and the lowest point, were found in *Salmonella* enteritidis (2) in comparison to the control group, without added radioactivity, by around 1.7 logs after 24 hours, slightly lower reduction was after 30 minutes-1.54 logs and the lowest at the end of the second day 0.74 logs. Lowest reduction was established in case of S. Enteritidis (1): by around 0.4 logs after 48 hours. It was observed that the levels of growth in all strains were slightly lower compared to strains that have been exposed to radioactivity from 1 kBq.

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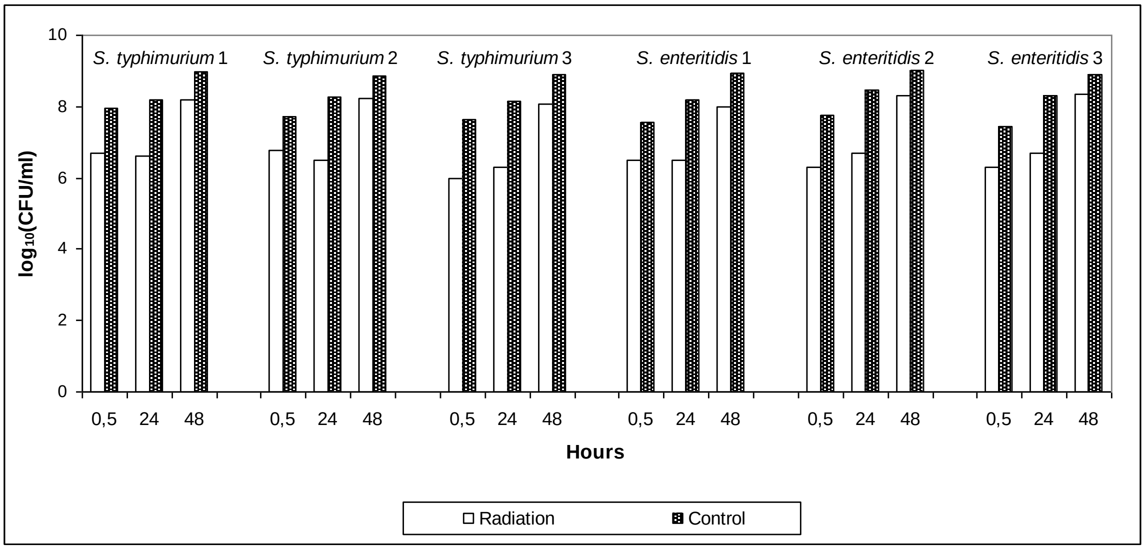


**Figure 3.** ***Salmonella*** **counts in inoculated meat homogenate without uranium (control IAFH) and with uranium of 5 kBq radioactivity (IAFH+U) during storage**

In Figure 3. the differences in *Salmonella* counts between pig meat homogenates with or without added radioactive uranium (5 kBq) are shown. Here, we see a slight increase differences salmonella counts immediately after 30 min. in comparison to the results in Table 2, especially for all three *S. Typhimurium* by around 1-1.7 logs. Difference between the *Salmonella* counts was the largest after first day (by around 1.6-1.8 logs). *Salmonella Typhimurium* (3) had the biggest differences by around 0.8 - 1.8 log. The results obtained after the second day show increase in differences between Salmonella counts of the control group with and without added radioactivity by around 0.6-1 log.

A constant increase of the reduction factor is observed. Salmonella tiphimurium 3 and *Salmonella enteritidis* (2) showed biggest differences between salmonella points without and with added uranium after 24 hours: for *Salmonella tiphimurium* (3) of 1.6 to 1.9 and for *S. enteritidis* (2) 1.6 to 1.8 logs. The values of the salmonella points after added uranium were lower in all three phases of measurement compared to Figure 2. The average ranged from 0.2 to 0.4 logs. Also, the reduction factors in both groups of salmonella after 48 hours were higher in reference to Figure 2. These are the values of the reduction factor ranging from 0.6 to 0.9 logs and were higher than the value of reduction factor after 48 hours in Figure 2, which ranged from 0.4 to 0.7 logs.

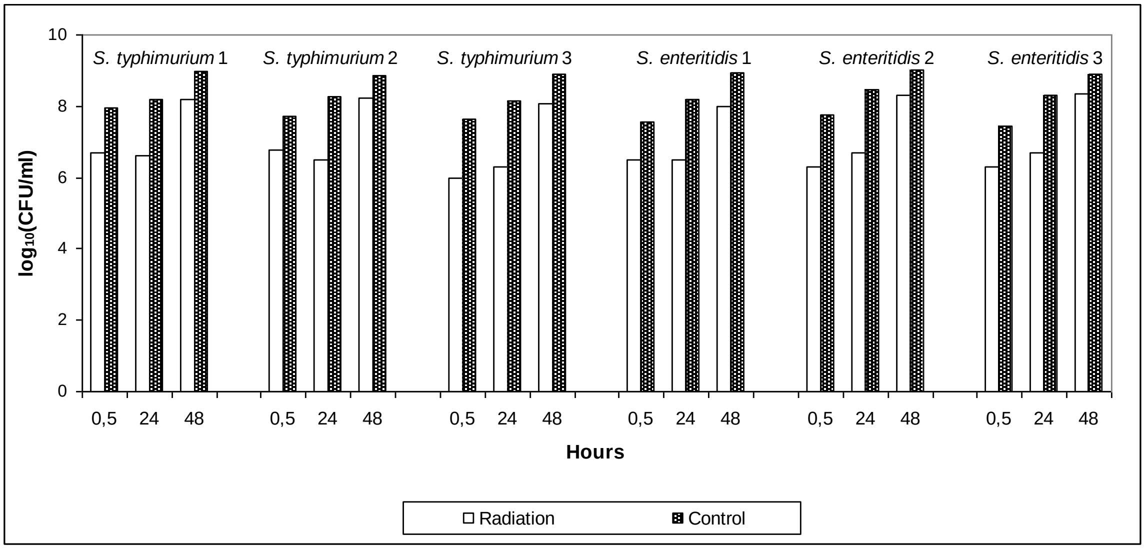


**Figure 4.** ***Salmonella* counts in inoculated meat homogenate without uranium (control IAFH) and with uranium of 10 kBq radioactivity (IAFH+U) during storage**

The highest dose of radioactivity was added to the fourth group of pig meat homogenate with uranium (10 kBq), as shown in Figure 4. At this stage of the experiment the biggest difference in salmonella counts between groups was reached. After 30 min the reduction was by around 1-1.7 logs. The greatest reduction of *S. Tiphimurium* (3) was 1.65 logs in relation to all six strains.

The level of reduction after first day was by around 1.6-2 logs *Salmonella tiphimurium* (3) had the highest reduction factor after 24 and 48 hours, which amounted to 1.96 logs that is 0.96 logs.

Also, after two days, the reduction was by around 0.7-1 logs. Greater reduction was observed in *S. Typhimurium* (3) with value of 0.96 logs.

Tendency of decrease in the value of salmonella counts in this phase of the experiment was the highest as compared to the previous three groups. Here, the *S. Typhimurium* (3) had the lowest point of growth compared to other strains in all three measurements.

It is likely that this reduction was due to the effect of added radioactivity, although the exact mechanism of the effect was neither investigated nor explained in the present stage of the study. Furthermore, as indicated above, the observed added radioactivity-induced reduction rates were more prominent in *S. Enteritidis* then in *S. Typhimurium*. However, the limited scope of this preliminary study was insufficient to identify either if this difference is serovar- or strain-related, or whether other genotypic or phenotypic characteristics of the pathogen were affected.

## 4. Conclusion

This preliminary study showed that all four levels of radioactivity can significantly reduce 6 *Salmonella* serovars in pork meat. The greatest reduction is obtained when the added radioactivity was 10 kBq.

Only two *Salmonella* serovars in all four levels of radioactivity show any deviations and significantly reduce the *Salmonella* counts in pork meat homogenates. This applies to *S. Typhimurium* (3) and *S. enteritidis* (2).

All three *S. enteritidis* shown in relation to a *S. Typhimurium* decrease the *Salmonella* counts when the radioactivity of 1 kBq and 2 kBq was added. This applies particularly to *S. Enteritidis* (2) where it was 1.6 logs to 1.7 logs.

When the radioactivity of 5 kBq and 10 kBq was added all three *S. Typhimurium* had greater reduction than *S. enteritidis*. This applies particularly to *S. Typhimurium* (3) where it was 1.8 logs to 1.9 logs.

In further studies, which are ongoing, these observations must be confirmed and the mechanisms causing these effects explained. Number of data on the content of radionuclides of the uranium and thorium series in feed, food and nutrition is small and the current results can hardly be used to determine their relations in all geographic areas. Therefore, in following period more attention should be turned to the content and transfer of natural radionuclides of uranium and thorium series in animal feeds, especially because the content of natural radionuclides in natural phosphates, which are used in feed for pigs, can reach very high values.

## 5. Acknowledgement

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