CASE REPORT

Epidemiological and epizootological aspects of salmonellosis

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Abstract

In their case report, most of the patients with salmonellosis mentioned consumption of meals containing poultry products, primarily eggs and egg products as a source of their disease. Microbiological analysis of samples showed that in more than 88 % of cases the infection agent was identified as Salmonella enteritidis. This serovar is the most frequent pathogen isolated at poultry farms. In the period of the past five years, 171 outbreaks of salmonellosis were recorded in Slovakia. However, within the group of animal tenders at these farms, no case of salmonellosis was confirmed. The alimentary character of salmonellosis led us to check results of analyses of samples of food and foodstuff of animal origin examined for Salmonella spp. performed during the past five years. The control of results indicated that out of these 228 545 samples of food and foodstuff of animal origin only 0.21 % were confirmed as being Salmonella positive and the average ratio of Salmonella enteritidis occurrence in samples was 0.1 % per year. A higher incidence (1.43 %) was recorded only in eggs and egg products. Our observations indicated that there was a change in tenacity of Salmonella enteritidis due to its increased resistance, primarily against elevated temperatures including that of pasteurization. An everyday requirement for decreasing the incidence of salmonellosis is based on strict hygienic behaviour "from stable to table". The objective of salmonellosis elimination strongly depends on amending the current legislation as to the establishment of hygienic conditions in complete food chains. ***(Tab. 4, Ref. 21.)***
Key words: salmonellosis, risk of transmission, domestic animals, food.

From the time of the discovery of the bacteria causing diarrhoea in pigs (named *Salmonella choleraesuis*) by B.D. Salmon D.V.M. (1884), bacterial strains of *Salmonella* became an object of interest in numerous studies. A lot of analyses of bacterial structure were performed to complete the microbiological profile of *Salmonella*. In 1914 only 14 strains of *Salmonella* were known all over the world. The current O.I.E. (Office Internationale des Epizooties) Manual defines 2375 serovars.

At the world congress about alimentary diseases, held in 1998 in Berlin, an opinion was accepted that as to gastrointestinal diseases campylobacteriosis was becoming even more important than salmonellosis. This suggestion was undertaken due to the increased rate of outbreaks of campylobacteriosis in Canada, France and Great Britain. Year after year, an increasing number of patients were recorded also in Czech Republic. The following years confirmed the same prognosis also in Slovak Republic.

A similar situation has developed in Slovakia, the number of patients increased, but in 1998 it stabilized on the level of 1400 cases per year and this level has been kept up to the present time.

The statistical analysis showed that salmonellosis would be the leader in gastrointestinal diseases also during the fortcoming decades. In Czechoslovakia, the official registration of salmonellosis outbreaks was started in 1951, and 1819 cases were reported at that time (Nižňanský et al, 1965). In Slovakia, reports in 1957 presented 288 treated persons, in 1989 the rate of patients increased up to 5683 cases and culminated in 1998 on the level of 21 287 reported cases of salmonellosis. Approximately 45 serovars were identified per year and more than 88 % were related to *Salmonella enteritidis*. The primacy of *Salmonella enteritidis* has been kept for nearly past 10 years whereas before it was being replaced with *Salmonella typhimurium*. Great at-

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Tab. 1. Salmonellosis in human population from 1998 to 2002 (Statistics of State Health Institute, Banská Bystrica, 2003).

Year	1998	1999	2000	2001	2002	Total
Rate of diseases	21287	18915	18143	19517	15854	93716
Men	10119	9115	8796	9405	8029	45464
Women		9800	9347	10112	78254	8252
Morbidity/100 000 humans	168	351	336	361	293	342
Carriers	806	614	516	604	549	3029
Serovars	49	55	40	33	45	55
S. enteritidis (%)	86.4	87.8	89.9	87	89.5	88.12
Mortality	1	-	1	2	1	5

Tab. 2. Occurrence of salmonelosis in animals (1998–2002).

Year	1998	1999	2000	2001	2002	Total
			Outbreaks			
Poultry	71	35	29	19	17	171
Cattle	4	8	8	5	8	33
Sheep	0	1	0	1	1	3
Pigs	12	22	15	19	13	81
Total	97	66	52	44	39	298
			Number of suffering animals			
Poultry	1543903	566526	370234	324652	206038	3011353
Cattle	88	519	588	190	3741	759
Sheep	0	14	0	9	9	32
Pigs	4483	3781	5125	4647	7616	25652
Total	1548474	570840	375974	329498	214498	3038796
			Number of perished animals			
Poultry	173626	88012	55657	33757	276093	78661
Cattle	33	117	134	41	80	405
Sheep	0	14	0	0	0	14
Pigs	17	696	985	939	1270	3907
Total 1	73666	88839	56776	34737	28959	382987

tention must be paid to carriers because in the period of the past 5 years, 3029 of them have been revealed (Statistics of State Health Institute, 2003).

Material and methods

In the period from 1998 to 2002, the monitoring of the occurrence of *Salmonella*, focused on the serovar of *Salmonella enteritidis* in food of animal origin, was evaluated. At this time also salmonellosis in domestic animals (poultry, cattle, pigs, and sheep) was monitored.

The statistical analyses of State Health Institutes were used as the background in searching the dependence between morbidity of consumers and occurrence of *Salmonella* in food and food products of animal origin.

Examinations of individual commodities of food were performed in regional laboratories of State Veterinary and Food Institutes, each analysing samples collected from their own attraction areas. The methods used were in compliance with standard procedures recommended by O.I.E.:

– cultivation and isolation of suspect colonies,

– biochemical identification of suspect colonies using BBL method ENTEROTUBE II preceded by the test of catalase activity, oxidase test and the proof of production of indole. The method and its computer coding and identification system (CCIS) are designed especially to identify the family of *Enterobacteriacea* — aerobic and facultative anaerobic, gram-negative and oxidase-negative rod-shaped bacteria. The diagram demonstrates how the oxidase test and BBL ENTEROTUBE II can be used to distinguish the family *Enterobacteriacea* from other gram-negative bacteria,

– as the same time a method according to STN ISO 6579 was used,

– individual serovars of Salmonella enteritidis were fagotypified according to the manual of producer LEEC LIMITED (Nottingham, Great Britain).

Tab. 3. Occurrence of salmonellosis in food and foodstuff of animal origin (1998–2002).

Year	1998	1999	2000	2001	2002	Total
Number of samples	58378	48506	43119	40293	38249	228545
Salmonella spp. (%)	0.16	0.16	0.17	0.37	0.19	∅0.21
S.enteritidis (%)	0.07	0.09	0.08	0.21	0.09	∅0.10
S.typhimurium (%)	0.01	0.005	0.01	0.01	0.05	∅0.017
S.derby (%)	0.001	0.001	0.001	-	0.02	∅0.057
S.montevideo (%)	-	0.04	0.07	0.08	0.02	∅0.052
Others (%)	0.8	0.02	0.01	0.07	0.01	∅0.18
			Commodities			
Eggs (%)	0.31	0.35	0.29	0.83	0.25	∅0.40
Egg products (%)	1.69	0.77	2.19	2.8	1.45	∅1.43
Poultry (%)	3.45	2.86	2.10	2.04	2.19	∅2.52
Poultry products (%)	1.54	0.75	0.59	0.48	0.61	∅0.79

∅ — average values

Tab. 4. Occurrence of individual PT of Salmonella enteritidis (1997–2003).

Year	PT												U	C	Total
	1	3	4	6	6a	7	8	9b	21	13a	21	23			
1997	87	-	16	-	-	-	110	-	-	-	-	1	29	7	250
1998	16	-	79	-	-	5	200	-	-	1	-	-	21	1	323
1999	21	1	135	7	5	6	81	1	-	-	4	8	18	14	301
2000	52	-	142	5	2	1	157	-	1	-	-	11	27	10	408
2001	40	2	109	2	-	1	169	-	1	-	-	9	14	7	354
2002	30	-	80	-	-	2	122	-	-	-	-	3	15	-	252
2003	28	-	50	-	-	-	64	-	-	-	-	1	10	-	153
Total	274	3	611	14	7	15	903	1	2	1	4	33	134	39	2041
%	13.4	0.2	29.9	0.7	0.4	0.8	44.2	0.04	0.08	0.04	0.16	1.6	6.58	1.9	100

PT — phagotypes, U — unknown phagotypes, C — contaminated samples

In the period of 5 years 228 545 samples of food and food products of animal origin were examined. Fagotypification was performed on 2041 strains of *Salmonella enteritidis*.

Results

298 outbreaks of salmonellosis in domestic animals were reported in Slovakia during the past 5 years. Outbreaks occurred in farms of sheep (1 %), cattle (11.07 %), pigs (27.18 %), and poultry (57.38 %) as described in Table 2.

At the same period health institutes recorded 93 716 cases of salmonellosis in humans and 3 029 cases of carriers without any clinical signs of diseases. The parenteral localisation of *Salmonella* was determined in 295 persons. *Salmonella enteritidis* was isolated in an average of 88.12 % of positive cases per year (Tab. 1).

During the evaluated 5 years 228 545 samples of food and food products of animal origin were examined for Salmonella with an average rate of positive cases on the level of 0.21 % per year and with 0.1 % ratio for *Salmonella enteritidis*.

Out of the monitored commodities the primary was found to be in poultry with the ratio of 2.53 %. *Salmonella* was isolated directly from 0.4 % of chicken eggs, however positive isolates were recorded in as many as 1.43 % of egg products (Tab. 3).

In the period of 7 years, 2 041 samples were analysed for of *Salmonella enteritidis* strains in our laboratory for phagotypification. 12 phages were identified, the most frequent type of phages was PT 8 (44.2 %) and PT 4 (29.9 %) and other phages did not achieve the level of 1 % (Tab. 4).

Discussion

In human population, the rate of serovars of *Salmonella* varies approximately on the level of 45, out of which in more than 88 % *Salmonella enteritidis* as dominantly involved. The primacy of *Salmonella enteritidis* has been kept for nearly 10 past years whereas before it was being replaced with *Salmonella typhimurium*. The location of Salmonella adapted to individual species of animals in sick humans and carriers is not significant.

No direct contact between human patients and animals contaminated with *Salmonella* was given in their case histories. This fact is related to the affinity of serovars to specific animal.

Salmonellosis affects all species of domestic animals, by serovars adapted to individual speciesy. The most sensitive are the young, pregnant as well as immunodefficient animals. After a short incubation period (usually 2–6 days), typical septicaemia develops, and in the newborns, depression, anorexia, weakness, fever, and death can ensue. In adult animals, an acute form of salmonellosis is accompanied by fever, thirst, anorexia, and diarrhoea, as well as frequent aborts. The chronic form of disease is characterised by diarrhoea, intermittent body temperature, anorexia and rarely also by arthritis, respiratory and nervous failures ensued by death. The chronic form is most frequent in pigs before their feeding period (Paulik, 1999).

The sources of outbreaks on the farm level are usually fodder, birds, pests and humans, but the most dangerous factor are latent carriers (Pipová et al, 1995; Cabadaj et al, 1997). The highest incidence of salmonellosis is at poultry farms, followed by pigs and rarely at cattle farms. The risk of *Salmonella* transmission from cats and dogs to humans is considered in various ways, Dow (1989) referred 6 cases of clinical salmonellosis characterised by gastroenteritis in cats. Similar results were obtained also by White et al (2002). Examination of rectal swabs from 187 dogs and 13 cats showed that *Salmonella* was present in only one dog (Kozák et al, 2003). Dahlinger et al (1997) isolated 3 strains of *Salmonella spp.* from mesenteric lymphatic nodes of 50 healthy dogs and Kallo and Hasso (2001) identified 7 serovars of *Salmonella* from rectal swabs of 150 dogs.

On the basis of these references, we can conclude that that dogs and cats did not play an important role in the transmission of salmonellosis.

A similar role in transmission is played by fish and cold-blooded animals. However, the presence of water poultry (ducks and gooses) in fish farms must be considered as a potentially dangerous factor. Faeces of poultry can be a source of salmonellosis in fish although growth of *Salmonella* is slowed down due to low temperature. In addition, fish are usually thermally treated before consumption.

298 outbreaks of salmonellosis in domestic animals were reported in Slovakia during the past 5 years. Outbreaks have occurred in farms of sheep (3), cattle (33), pigs (81), and poultry (171) as described in Table 2.

Microbiological examinations of food are under control of veterinary institutes, but the financial budget assessed for sample analysis is limited and the number of samples decreases year by year (for instance 86 283 samples of food and foodstuff of animal origin in 1998 and 40 293 samples in 2001). Findings of salmonellosis were stabilized at 0.21 % (Tab. 3), the most frequent was that of *Salmonella enteritidis*, followed by *S. typhimurium, S. derby, S. montevideo* etc. *Salmonella* was isolated directly from 0.4 % of chicken eggs, however, positive isolates were recorded in as many as 1.43 % of egg products (Tab. 3). An opposite ratio was showing in poultry and poultry products (2.52 % and 0.79 %).

Modern industrial technologies of processing of slaughter poultry represent also some risks. Among thousands individuals of slaughtered poultry some are usually found to be contaminated by latent form of salmonellosis thus introducing a hazard of cross contamination (Turek et al, 1989; Ivanová et al, 1995). Critical points are represented by scalding tanks and chilling basins where water contaminated by faeces transmitts the infections agent to the surface of other pieces. This is the reason why the incidence of salmonellosis in poultry is so high, including frozen poultry (Saladiová et al, 2001; Nagy a Nagyová, 2003). Thawing of poultry meat requires some times and kitchen temperature in combination with time represent an optimal condition for the growth of *Salmonella*. Contamination of hands and utensils (knives, taps, etc.) is followed and together with improper hygiene (cleaning, washing, disinfecting) represent an opportunity for *Salmonella* to reproduce and be transmitted in frame of family circulation.

Poultry meat and offal is considered as fit and safe for human consumption after being boiled, grilled or thermally treated.

Statistical records of hospitals described in case histories of patients as the most frequent source of salmonellosis are consumption of meals and egg-containing products. This factor of transmission is recorded in patients with confirmed as well as suspect diagnoses.

The occurrence of *Salmonella* in vehiculum and on the eggshell is not as frequent as it is expected. Poppe (1996) referred in his paper that at farms contaminated by salmonellosis the ratio of vertical transmission is one transovarial infected egg per 1000 and horizontal transmission is more frequent. Egg white and yolk are suitable media for growth and reproduction of *Salmonella*, especially at optimal temperature conditions. Home celebrations and restaurants represent a higher level of risk because the possible occurrence of *Salmonella* in eggs can affect a huge number of consumers. One infected egg can cause reproduction of *Salmonella* (1 000 000 bacteria per 6 hours) thus increasing the incidence of consumers morbidity (30 %). This is one of explanations as to why the incidence of salmonellosis is 4 times higher in summer months than in winter time. Similar results were obtained also by Cabadaj et al (1995) and Pipová et al (2001).

Incomplete accompanying documents of samples assessed for phagotypification of *Salmonella enteritidis* and non traceable transport of animals and commodities are the reasons why we are not able to recognise the dependencies of occurrence of individual PT. A similar problem was described by Gast and Benson (1995), Halavatkar and Barrow (1993).

Our results and conclusions of Notermans et al (1996) and Ziedler et al (1996) predicted that the elimination of salmonellosis might take a long time. The establishment of strict hygienic behaviour in complete chain of poultry production and processing of poultry products must be accepted (from stable to table). Enforcement of national legislation, primarily governmental regulations is required (in accordance with EU legislation):

– on hygiene and health problems affecting the production and placing on the market of egg products (Governmental regulation No 272/2003)

– on hygiene and health problems affecting the production and placing on the market of fresh poultry meat (Governmental regulation No 282/2003).

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