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Serological diversity and antimicrobial resistance of *Salmonella* isolated from different sources in Lithuania

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ABSTRACT

Over 57000 samples were tested in order to isolate *Salmonella* from animals and feedstuffs during the period 1998-2003. Four hundred and nine strains were isolated from poultry, 143 from pigs, 16 from cattle and 99 from feedstuffs. Ninety-two percent of isolated *Salmonella* from pigs depended to serovar *S.* Choleraesuis. All *Salmonella* isolates from cattle depended to serovars *S.* Dublin and *S.* Enteritidis. A wider variety of *Salmonella* serovars was obtained from poultry and feedstuffs. The main source of *Salmonella* presence in feedstuffs was fish products. Sixty isolates of *S.* Choleraesuis and 100 isolates of different serovars from poultry were tested for antimicrobial resistance. Only 1.3% of isolated *Salmonella* were resistant to ceftiofur, 5.6% to enrofloxacin. Thirty-four percent were resistant to nalidixic acid, 33% to tetracyclines and 8.1% were resistant to aminoglycosides (neomycin and gentamicin). Streptomycin was less effective: 30% of isolated *Salmonella* were resistant to this antibiotic. *S.* Choleraesuis has more resistance than *Salmonella* serovars isolated from poultry. There is evidence that the prevalence of *Salmonella* has decreased in recent years. However, more attention must be paid to surveillance and monitoring of antimicrobial resistance.

Key words: *Salmonella*, poultry, pigs, feedstuffs, antimicrobial resistance

Introduction

Salmonella is found worldwide in poultry, pigs and cattle and is considered the most important animal related zoonoses today (ANDERSON et al., 1999; LO FO WONG, 2002). Healthy and sick animals can carry a wide range of *Salmonella* serotypes and can be a

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source of contamination throughout the meat production and processing systems (STEGE et al., 2000; PRUKNER-RADOVČIĆ and CIGLAR GROZDANIĆ, 2003; LO FO WONG et al., 2004). Salmonellosis is one of the most important zoonosis in Lithuania. *Salmonella* infections are detected in poultry flocks, pigs and cattle farms every year. It is also found in feedstuffs. European Union member states follow the Directive of Zoonoses 92/117EEC (currently it was amended by 2003/99/EC) imposing the gathering of epidemiological data concerning the number of animal salmonellosis cases and prevalence of isolated *Salmonella* serovars. A national surveillance and control programme was implemented in Lithuania. This programme is based on the *Salmonella* control act enforced by the Ministry of Agriculture. The main component of the programme contained *Salmonella* monitoring in poultry flocks.

The goals of the present study are associated with the investigations in the epidemiological situation of *Salmonella* infections found in domestic animals and feedstuffs. Determination of antimicrobial resistance of isolated *Salmonella* strains constitutes another important issue of this work.

Materials and methods

The studies were carried out at the National Veterinary Laboratory (NVL) and Veterinary Institute of the Lithuanian Veterinary Academy (VILVA). Investigations were carried out using pathological and clinical material of poultry, pigs, cattle and feedstuffs. *Salmonella* isolation and identification procedures followed ISO 6579 "Microbiology - general guidance on methods for the detection of *Salmonella*". Buffered peptone water (Oxoid, CM509) was used for the initial detection of *Salmonella*. Rappaport Vassiliadis Medium (Oxoid, CM669) was used for the selective enrichment. Isolation of *Salmonella* was carried out using XLD Medium (Oxoid, CM469), SS Agar (Lab M, Lab52) and Brilliant Green Agar (Oxoid, CM263). Reference standard bacterial strains were used as controls. Identification covered 667 *Salmonella* strains from poultry (61%), pigs (21%), cattle (2.4%) and feedstuffs of animal origin (15%). Biochemical testing was performed using the biochemical system "Crystal" (BBL, USA). In more complicated cases classical methods were applied.

Serological testing was performed according to generally accepted rules by a slide agglutination test according to the Kauffmann-White scheme (POPOFF, 2001).

The agar diffusion method according to NCCLS guidelines (NCCLS, 1997) was applied for antimicrobial susceptibility testing of *Salmonella* isolates. Mueller Hinton Agar II (BBL, LAB39) was used in order to perform this test. Suspension of 0.5 McFarland unit density was used for inoculation. One hundred and sixty animal isolates (100 from poultry and 60 from pigs) were tested for antimicrobial susceptibility. The following antimicrobial agents (BBL) were used: ampicillin (10 μg), streptomycin (10 μg), tetracycline (30 μg), gentamicin,

(10 µg), neomycin, (10 µg), nalidixic acid (30 µg), enrofloxacin (10 µg), ceftiofur (30 µg), sulphametoxazole/trimethoprim (23.75 µg + 1.25 µg), chloramphenicol (10 µg) and colistin (10 µg). The results were interpreted by special manufacturer's tables.

Statistical analysis was performed using the programme Sigma Plot (Jandel Scientific, version 1.02a). A value of $P \leq 0.05$ was considered to be significant.

Results

Over 57000 samples were tested for *Salmonella* isolation during the period 1998-2003. The percentage of positive results ranged from 0.8% in feedstuffs, to 14% in samples originating from pigs. Results of *Salmonella* isolation from different sources are presented in Table 1. Four hundred and nine *Salmonella* strains originated from poultry, containing 61% from all *Salmonella* isolates. Twenty-one percent of all *Salmonella* isolates originated from pigs, 15% from feedstuffs and 2.4% from cattle.

Table 1. Isolation of *Salmonella* from different sources during the period 1998-2003

Samples tested		Number (%) of *Salmonella* positive samples
Origin	Number	
Cattle	259	16 (6.2)
Pigs	990	143 (14)
Poultry	43 350	409 (0.9)
Feedstuffs	12 600	99 (0.8)
Total 57 199		667 (1.2)

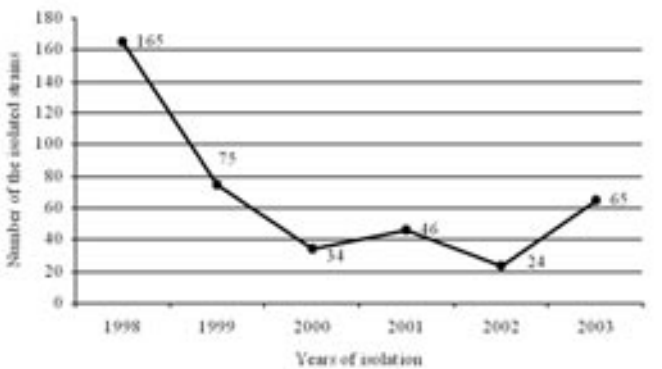

Fig. 1. Dynamics of *Salmonella* prevalence in poultry

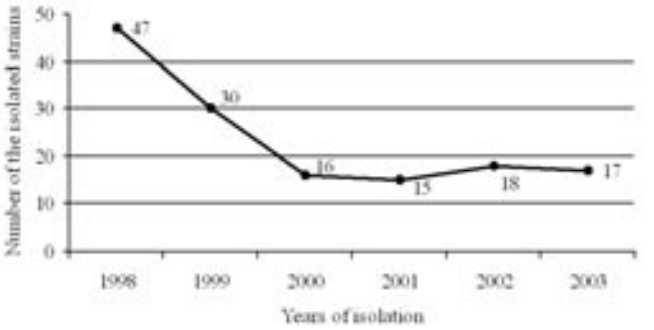

Fig. 2. Dynamics of *Salmonella* prevalence in pigs

Dynamics of *Salmonella* prevalence in poultry are presented in Fig 1. As can be seen, in 1998 165 *Salmonella* strains were isolated from poultry, containing 40% of all *Salmonella* strains isolated from poultry in the period of investigations. Seventy-five *Salmonella* strains from poultry were isolated in 1999, 34 in 2000, 46 in 2001, 24 in 2002 and 65 strains in 2003.

Dynamics of *Salmonella* prevalence in pigs are presented in Fig. 2. As can be seen, in 1998, 47 *Salmonella* strains were isolated, containing 33% of *Salmonella* isolates originating from pigs. Thirty *Salmonella* isolates were isolated in 1999, 16 in 2000, 15 in 2001, 18 in 2002 and 17 strains in 2003.

Fig. 3 shows the sources of *Salmonella* prevalence in different types of feedstuff. As can be seen, more than a half of isolated *Salmonella* (64%) were isolated from fish products.

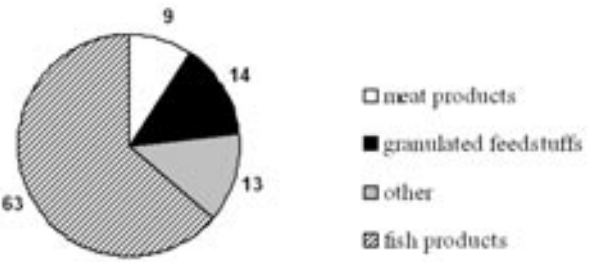

Fig. 3. Main sources of *Salmonella* spread in different types of feedstuffs (%)

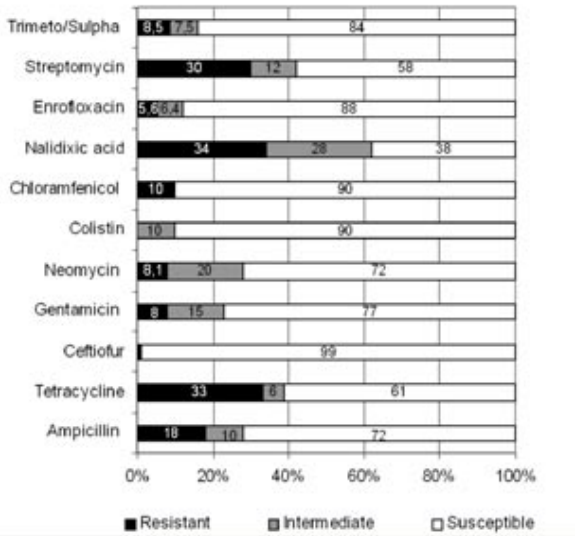

Fig. 4. Susceptibility of isolated *Salmonella* to antimicrobials (160 isolates)

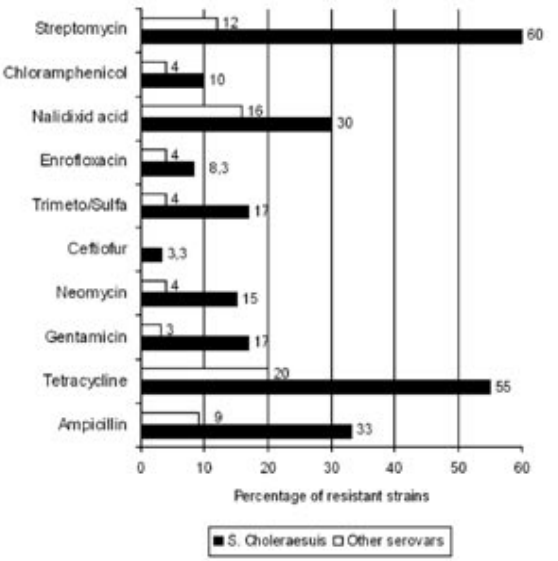

Fig. 5. Comparable resistance of *S.* Choleraesuis (pigs) and other serovars (poultry)

Table 2. Prevalence of *Salmonella* serovars according to source of isolation

Salmonella serovars	Source of isolation			
	Cattle	Pigs	Poultry	Feedstuffs
Agona				7
Choleraesuis		131		
Coeln				1
Derby				16
Djugu				1
Enteritidis	4		294	6
Dublin	12			
Eschweiler			1	7
Gallinarum			2	
Hadar			3	
Heidelberg			18	
Indiana				1
Infantis		6	4	2
Typhimurium			28	8
Virchow		3		1
B gr. (04)				1
C gr. (07)			19	1
D gr. (09)			2	
R form		3	8	14
unknown			30	33
Total	16	143	409	99

Prevalence of *Salmonella* serovars depends on the source of isolation. A list of *Salmonella* serovars, according to the source of isolation, is shown in Table 2.

Data on *Salmonella* susceptibility to antimicrobials is showed in Fig. 4. None of the used antimicrobials was fully efficacious against all tested strains. Ten per cent of tested *Salmonella* were intermediately susceptible to colistin, but there were no strains resistant to this antibiotic. Only 1.3% of *Salmonella* were resistant to ceftiofur. The resistance to fluorochinolones was low: 5.6% of isolated *Salmonella* were resistant to enrofloxacin. Resistance to the oldest generation chinolones (nalidixic acid) was 34%. Resistance to

tetracyclines was also high (33%). Resistance to aminoglycosides was 8.1%, except streptomycin (30%).

Differences of resistance between *S.* Choleraesuis and other tested serovars isolated from poultry are presented in Fig. 5. *S.* Choleraesuis is shown to be more resistant to all tested antimicrobials. More than a half of *S.* Choleraesuis isolates were resistant to tetracyclines and streptomycin and therefore appears to be more resistant than other serovars ($P \leq 0.05$). The same statistical situation existed among other antimicrobials - ampicillin, sulfamethoxazole-trimethoprim, aminoglycosides. It is necessary to emphasize that a large part of *S.* Choleraesuis isolates (30%) were multiresistant, i. e. showed resistance to three or more antimcrobials (data is not shown).

Discussion

The obtained results indicate that poultry was the major source of *Salmonella* isolation. The situation in other countries shows that *Salmonella* is more common in poultry (ANGULO and SWERDLOW, 1998; BÄUMLER et al., 2000; HOSZOWSKI and WASYL, 2002). Our investigations show that salmonellosis is infrequent in cattle but is more common in pigs. Cases of *Salmonella* isolation from other animal species are uncommon. However, there are data from other countries on *Salmonella* isolation from horses, fur and exotic animals. These animals tend to be *Salmonella* carriers (HOSZOWSK'Y et al., 2000). Animal feedstuffs, particularly fish products, often tend to be contaminating. It therefore causes a potential risk of salmonellosis transmission to food animals (JONES et al., 1982; BAGGESEN and AARESTRUP, 1998; HOSZOWSKY and WASYL, 2001). Not all animals infected by *Salmonella* fall sick; most of them stay carriers and excrete *Salmonella*s into the environment. Due to this reason the eradication of salmonellosis is not always successful.

There are more than 2500 *Salmonella* serovars (POPOFF, 2001). But only a few of them have clinical importance and are distributed worldwide. Our data show that the most widespread serovar in pigs was *S.* Choleraesuis, in poultry *S.* Enteritidis, and *S. Dublin* in cattle. *S.* Typhimurium is not very frequent in Lithuania although it was more frequent 15-20 years ago. For example, in 1985 91% of isolated *Salmonella* from poultry belonged to serovar *S.* Typhimurium. There are some specific aspects of *Salmonella* serovars prevalence in Lithuania. Ninety-six percent of isolated *Salmonella* from pigs belonged to serovar *S.* Choleraesuis. In other countries this serotype is infrequent and is replaced by *S.* Typhimurium (DAVIES et al., 1998; LETELLIER et al., 1999; GEBREYES et al., 2000). However, in certain countries, particularly those in Eastern Europe, *S.* Choleraesuis is frequent. For example, in 2001 in Poland, 58% isolated *Salmonella* from pigs belonged to *S.* Choleraesuis. This serovar is frequent and important in the U.S.A., where more than 90% of diagnosed salmonellosis in pigs is attributed to infections of *S.* Choleraesuis (ANDERSON et al., 1999). Salmonellosis caused by this serotype is primarily manifested as

a post-weaning septicaemia or enterocolitis and often occurs on operations that co-mingle pigs of different ages (SCHWARTZ, 1991). *S.* Choleraesuis is rarely isolated from sources other than pigs, which suggests an important role of carrier pigs in the spread of this host-adapted pathogen (SCHWARTZ, 1991; GRAY et al., 1996a; GRAY et al., 1996b). This serovar spreads clonally through horizontal and vertical routes of transmission in animals as well as through the contaminated feedstuffs (FEDORKA-CRAY et al., 1995; HARRIS et al., 1997; DAVIES et al., 1999). Feedstuffs are contaminated by different *Salmonella* serovars due to being imported from different regions.

The programme of salmonellosis observation and control, according to Directive 92/117/EEC, in poultry flocks commenced in 1996. According to this programme all the largest poultry farms were observed and controlled. Certain measures on salmonellosis control were taken in respect to other domestic animals - cattle and pigs. As a result of these measures, *Salmonella* isolation strongly decreased. Figs. 1 and 2 clearly demonstrate this fact. It is necessary to note that the impact of *Salmonella* reduction does not only comprise control and eradication measures, but also certain other reasons, such as essential changes in animal welfare, sanitary status in animal farms, which was reformed according to EU requirements. Reducing the number of some animals, particularly cattle and pigs, was also one of the most important factors in decreasing *Salmonella* infections. Over the last ten years the epidemiological situation changed in poultry and pig farms. New feeding technologies, new additives and drugs have had an impact on the ecological balance between microorganisms and macro-organisms. *Salmonella* is one of the most conformist bacteria, which is able to survive in various conditions. It is therefore impossible to displace them from the ecological chain by the extensive usage of antimicrobials. Antimicrobial resistance became a research topic and attracted great attention (STERNBERG, 1999; RUZAUSKAS and ZIENIUS, 2002; SKOWRON, 2003).

There were some data obtained earlier by us and by other authors on *Salmonella* prevalence in Lithuania (VIRGAILIS, 2000; RUZAUSKAS, 2002). However, there were no systemic data about antimicrobial resistance of *Salmonella* of any kind. According to our data, the level of resistance of *Salmonella* depended on the animal species or serovar. These data confirms those obtained by other authors (WASYL and HOSZOWSKI, 2001). For example, in Poland as well as in Lithuania *Salmonella* isolates from pig origin are more resistant than those from other sources. This can be explained by the uncontrolled usage of various antimicrobials in pig farms. The antimicrobials are given to piglets from the beginning of their lives as a prophylaxis measure. Broad-spectrum antimicrobials are often used without results of clear diagnosis or susceptibility testing. There are the factors, therefore which favour the formation of multi-drug-resistant bacteria strains. It has already been noted that *Salmonella* isolates obtained from pigs could express the resistance between some antimicrobials much more easily than poultry isolates (SEYFARTH et al., 1997; GEBREYES et al., 2000). However, there are certain data showing that resistance in pig isolates is

significantly lower, especially in such countries where pig breeding is less developed than in other branches of husbandry. For example, in the U.S.A. (Alberta) the resistance of *Salmonella* bovine isolates to ampicillin, tetracycline, streptomycin and sulfamethoxazole ranged from 60% to 69% and were the most resistant among the all isolates (JOHNSON et al., 2003). According to these data it may be underlined that development of resistance to antimicrobials mostly depends on the extensive use of antimicrobials.

A threatening situation exists according to the resistance to quinolones around the world. Thirty-four percent of the isolated *Salmonella* were resistant to nalidixic acid. Fluorochinolones were active against most *Salmonella* isolates. However, 5.6% were resistant to enrofloxacin. Such a fact proves that *Salmonella* strains spread in Lithuania have accomplished the first step in quinolone resistance development - single-point gyrase gene. The tendency indicates the increasing resistance to fluoroquinolones. Additionally, other authors from different countries confirm such a tendency (HOSZOWSKI and WASYL, 2002; PIDDOCK, 2002).

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SAŽETAK

Više od 57000 uzoraka pretraženo je u svrhu izdvajanja bakterija roda *Salmonella* iz životinja i krmiva u razdoblju od 1998. do 2003. god. Iz peradi je izdvojeno 409 izolata, 143 iz svinja, 16 iz goveda i 99 iz krmiva. Devedeset i dva posto (92%) izdvojenih salmonela iz svinja pripadalo je serovaru *Salmonella* Choleraesuis. Svi izolati iz goveda pripadali su serovarovima *S*. Dublin i *S*. Enteritidis. Širi raspon serovarova uočen je u peradi i krmivima. Glavni izvor salmonela prisutnih u krmivima bili su riblji proizvodi. Šezdeset izolata *S*. Choleraesuis i 100 izolata različitih serovarova iz peradi bilo je pretraženo na otpornost prema antimikrobnim tvarima. Samo je 1,3% izolata bilo otporno na ceftiofur, a 5,6% na enrofloksacin. Trideset i četiri posto bilo je otporno na nalidiksičnu kiselinu, 33% na tetracikline i 8,1% na aminoglikozide (neomycin i gentamicin). Streptomicin je bio manje djelotvoran te je 30% izdvojenih salmonela bilo otporno na ovaj antibiotik. *S*. Choleraesuis je bila otpornija od serovarova izdvojenih iz peradi. Činjenica je da se proširenost salmonela smanjuje posljednjih godina te da više pozornosti treba posvetiti nadzoru nad antimikrobnom rezistencijom.

Ključne riječi: salmonele, perad, svinje, krmiva, antimikrobna otpornost