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Integrated veterinary networks for the surveillance of zoonotic agents in Italy

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Foodborne zoonoses are infections and diseases naturally transmissible between animals and humans via contaminated foodstuffs. The severity of these diseases in humans varies from subclinical infection or mild symptoms to life-threatening conditions. In order to prevent such zoonoses from occurring, it is important to identify which animals and foodstuffs are the main sources of infection. For this purpose, information aimed at protecting human health is collected and analysed from all European Union Member States. The European Union (EU) system for the monitoring and collection of information on zoonoses is based on the Zoonoses Directive 2003/99/EC, which obliges EU Member States to collect relevant and, where applicable, comparable data on zoonoses, zoonotic agents, antimicrobial resistance and foodborne outbreaks. According to the data reported in the latest European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks, published by EFSA in 2014, *Salmonella* remained the most frequently reported cause of foodborne outbreaks in the EU in 2012 (1,533 of 5,363 foodborne outbreaks, 28.6%).

In 2012, a total of 91,034 confirmed cases of human salmonellosis were reported in the EU. This represents a decrease of 4.7% compared with 2011 and a decrease of 43,546 cases (32%) compared with the case numbers reported in 2008. The EU notification rate for confirmed cases was 22.2 cases per 100,000 population. The EU case-fatality rate was 0.14%, as 61 deaths due to non-typhoidal salmonellosis were reported in the EU in 2012. As in previous years, S. Enteritidis and S. Typhimurium were the most frequently reported serovars (41.3% and 22.1%, respectively, of all known reported serovars in human cases); monophasic S. Typhimurium 1,4,[5],12:i:- was the third most commonly reported serovar in the EU (7.2%). The fourth most common serovar in humans was *Salmonella* Infantis, with increasing numbers of reported isolates over the last five years (EFSA, 2014).

Knowledge about strain characteristics, through the typing of isolates, is of paramount importance in order to investigate sources of disease by comparing strains isolated from different reservoirs. The harmonised serotyping system is crucial in establishing the epidemiology of *Salmonella* at all levels. Ideally, a large proportion of, if not all, *Salmonella* isolates from humans, animals and food should be subtyped, to support epidemiological insight and to target interventions to prevent infections from identified sources (Wagenaar *et al.,* 2013). Information is needed about the presence of pathogens in the full array of exposure sources (e.g., foods, animals, drinking water, and recreational water) (Parmley *et al.,* 2013). Integrated surveillance programmes collect samples and generate information from multiple components within a system (Galanis *et al.,* 2012).

Microbial subtyping is one of the major methodologies to attribute food-borne infectious diseases to their sources. The principle behind source attribution is the comparison of subtypes of isolates causing human disease with the distribution of these subtypes in their putative sources (e.g. animals, food, or the environment) (Pires *et al.,* 2009). Microbial subtyping source attribution relies on laboratory subtyping methods to identify overlaps between subtypes identified from cases of human disease and those from their potential sources (Barco *et al.,* 2013).

Human infections caused by subtypes that have been exclusively or almost exclusively isolated from a single source can then be attributed to that specific source. On the other hand, when human infections are caused by subtypes isolated in several sources, they can be attributed to those sources proportionally to the reported occurrence of subtypes in the sources (Hald *et al.,* 2004).

In Italy, in order to collect data about *Salmonella* strains isolated in the veterinary sector, the Enter-Vet network was established in 2002, with the aim of collecting data at the national level on *Salmonella* spp. detection from samples of animal origin. This network operates in close collaboration with the Enter-Net system, which manages data on strains of human origin.

The Enter-Vet network consists of the laboratories of Istituti Zooprofilattici Sperimentali (IZS) under the supervision of the National Reference Laboratory for *Salmonella* (NRL). The Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe) was designated as the National Reference Laboratory for *Salmonella* in 1999 by the Ministry of Health. In 2007, the laboratory was recognised by the World Organisation for Animal Health (OIE) as a Reference Laboratory for salmonellosis. The laboratories participating in the Enter-Vet network send to the NRL data on *Salmonella* spp. strains serotyped, together with *Salmonella* Enteritidis, *Salmonella* Typhimurium and the monophasic variant of *Salmonella* Typhimurium isolates to be phage typed. Serotyping is carried out by all laboratories of the network according to the Kauffman-White scheme, while phage typing is performed by the National Reference Laboratory according to the schemes provided by the Health Protection Agency (Colindale, London, UK).

In order to guarantee the quality of the data produced by the network, the NRL organises annual ring trials for the laboratories of the network, as external quality control for *Salmonella* serotyping and detection. The NRL publishes an annual report of Enter-Vet activity, which can be downloaded from the IZSVe website (www.izsvenezie.it). The reports give an overview on

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the distribution of *Salmonella* serotypes and phage-types in different animal species and food sources at the national level, providing information useful for targeting epidemiological investigations in cases of human outbreaks. Moreover, all the strains are properly stored as well as the related epidemiological information, making it possible to perform more accurate typing, also through molecular methods, on subsets of strains that appear to be relevant at a certain time.

In 2012, the network collected information on 3,567 *Salmonella* strains, 58% of them isolated from animals at primary production, 30% from food matrices, and 4% from feeding stuffs and environmental samples (mostly at farm level). Poultry and swine are the most represented animal species. **Table 1** reports the distribution of serotypes in the different sources.

Table 1: serovars isolated from different sources, Enter-Vet, 2012

Serovar	Animal	Food	Animal feed	Environment	Unknown	Water	Total	%
Monophasic variant S. Typhimurium	237	266	2	27	3	2	537	15.05
Typhimurium	199	92	3	6	10	5	315	8.83
Derby	95	166	2	5	0	2	270	7.57
Livingstone	144	19	15	8	2	1	189	5.30
Infantis	59	53	1	7	0	0	120	3.36
Rissen	37	71	3	1	0	1	113	3.17
Thompson	90	13	2	5	1	0	111	3.11
Enteritidis	59	11	0	19	2	0	91	2.55
Agona	43	6	32	6	2	1	90	2.52
Mbandaka	57	4	16	3	5	0	85	2.38
Bredeney	51	27	2	2	1	0	83	2.33
Kentucky	71	7	0	5	0	0	83	2.33
Hadar	35	23	0	1	0	4	63	1.77
Veneziana	52	5	0	1	0	5	63	1.77
Muenchen	27	23	0	9	0	1	60	1.68
London	31	23	1	1	2	0	58	1.63
Braenderup	48	3	1	2	2	1	57	1.60
Coeln	52	3	0	2	0	0	57	1.60
Newport	32	19	2	1	0	2	56	1.57
Choleraesuis	45	3	0	0	0	0	48	1.35
Blockley	30	11	1	0	5	0	47	1.32
Saintpaul	16	21	0	1	0	0	38	1.07
Give	10	16	0	3	0	1	30	0.84
Anatum	14	11	3	1	0	0	29	0.81
Other serovar	349	143	60	27	14	18	611	17.13
Other	200	43	9	6	1	4	263	7.37
Total	2083	1082	155	149	50	48	3567	100.00

Surveillance is critical to the policy-making process, providing evidence needed to target interventions to improve food and water safety, and ultimately reduce the burden of disease. Laboratory surveillance, of which the Enter-Vet network is an example, is one of the pieces of this puzzle, whose probability of success in identifying the sources of disease, and therefore applying effective control measures, is a function of the level of integration within the system.

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