

Detection of *Listeria monocytogenes* in food samples in Avezzano, Sulmona and Castel di Sangro (province of L'Aquila, Abruzzo, Italy) between 2000-2009

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Keywords

Abruzzo,
Food categories,
Foodborne diseases,
Listeria monocytogenes.

Summary

The retrospective study of the results of the analysed samples is a fundamental tool for the identification of major risk related to food and for planning future monitoring activities. The evaluation of the quality of data collected may also allow for estimating the effectiveness of the controls so to improve their efficacy. In this article, the authors evaluated the results of tests for the detection of *Listeria monocytogenes* performed by the Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise 'G. Caporale' (IZSAM) on food samples collected during the years 2000-2009 in the territory of Avezzano, Sulmona and Castel di Sangro (province of L'Aquila, Abruzzo, Italy). The comparison of the data examined with those from studies conducted in Italy and in other countries shows that the categories with higher percentages of positivity for *Listeria monocytogenes* are meat and fish products. Data collected do not indicate cheese as a vehicle of contamination in the sampled areas, in contrast to what reported in the national and international literature. It would therefore be necessary to promote an *ad hoc* sampling in the areas covered by this study to verify this aspect in more depth.

Ricerca di *Listeria monocytogenes* in campioni di alimenti prelevati nel periodo 2000-2009 nei comuni di Avezzano, Sulmona e Castel di Sangro, provincia di L'Aquila, Abruzzo, Italia

Parole chiave

Abruzzo,
Categorie alimentari,
Tossinfezioni alimentari,
Listeria monocytogenes.

Riassunto

Lo studio retrospettivo dei risultati delle analisi dei campioni di alimento rappresenta uno strumento fondamentale per l'individuazione dei principali prodotti a rischio e per la pianificazione delle future attività di controllo nel territorio. La valutazione della qualità dei dati raccolti, inoltre, può permettere di stimare l'efficacia dei controlli precedentemente attuati, in prospettiva di un successivo miglioramento. Nel presente lavoro gli autori hanno valutato i risultati degli esami per la ricerca di *Listeria monocytogenes* effettuati dall'Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale" (IZSAM) su campioni di alimento prelevati nel decennio 2000-2009 nei comuni di Avezzano, Sulmona e Castel di Sangro in provincia di L'Aquila, Abruzzo, Italia. Dal confronto dei dati riportati in questo lavoro con quelli provenienti da studi effettuati in Italia e in altri Paesi si evince come gli alimenti con maggiori percentuali di positività per *Listeria monocytogenes* siano i prodotti carni e ittici. I dati rilevati dal presente lavoro, inoltre, non evidenziano un ruolo dei formaggi come veicolo di contaminazione del microrganismo nei territori oggetto di campionamento, a differenza di quanto riportato in letteratura nazionale e internazionale. Sarebbe necessario, pertanto, promuovere un campionamento *ad hoc* nei territori interessati dal presente studio mirato a verificare questo aspetto.

Introduction

Listeria monocytogenes is the causative agent of listeriosis, a serious disease that affects mostly infants, pregnant women and patients with immunosuppressive diseases causing septicaemia and meningitis in adults, abortions and stillbirths in pregnant women (Cordano and Rocourt 2001, Rantsiou et al. 2012). In the early stages, the infection has nonspecific symptoms such as fatigue, headache, joint and muscle pain, gastroenteritis. Without appropriate antibiotic treatment, however, it can develop into an invasive form characterized by septicaemia, meningitis, encephalitis and death (Vitas and Garcia-Jalon 2004). The incubation period is highly variable and can range from 3 to 70 days. The virulence of *L. monocytogenes* is associated with the individual sensitivity (Conter et al. 2007) and various characteristics of the strains including the serotype. Four (1/2a, 1/2b, 1/2c, and 4b) of the 13 serotypes identified and described in literature are responsible for the majority of clinical cases in humans. The 4b serotype is the most frequently isolated from outbreaks (Cordano and Rocourt 2001, Norrung et al. 1999). According to the results of the considered studies, long periods of adaptation to environmental stresses (changes in temperature, pH, aw) due to the intrinsic characteristics of certain foods may affect the virulence of this organism (Ryser and Marth 1999)¹.

L. monocytogenes causes disease in humans with an incidence ranging between 0.1 and 11.3 cases per million inhabitants. In Europe, the incidence is 3.5 cases per million inhabitants, while, in the United States of America and Australia, the incidence is 3 and 4.4 cases per million inhabitants, respectively (Nuvoloni et al. 2006, Mena et al. 2004, Uyttendaele et al. 1999, Farber and Peterkin 1991). Nonetheless, the severity of the disease and the associated high mortality (20-30%) justifies the interest devoted to it in terms of public health (Olesen et al. 2008). Listeriosis is generally considered a foodborne disease, due to the consumption of contaminated food. In fact, *L. monocytogenes* has been isolated in many aliments of animal origin (milk, cheese, ice cream, meat, meat products, fishery products) and in processing environments (Gattuso et al. 2005, Scallan et al. 2011)². In cooked products, the contamination can occur after the heat treatment or may be the consequence of an insufficient heating process. Ready to eat (RTE) foods are most frequently implicated in

outbreaks. Particularly, 3 categories of RTE foods have been identified as most at risk for consumers (Farber and Peterkin 1991): meat products subjected to heat treatment and subsequently manipulated (already portioned meat products, delicatessen products), soft or semi-soft cheeses (gorgonzola, taleggio cheese, brie, queso fresco) and smoked fish (salmon). Even raw milk, ricotta and different types of fruit and vegetables have been identified as cause of listeriosis outbreaks (Farber and Peterkin 1991, Conter et al. 2007, Olesen et al. 2008).

In accordance with the provisions of EC Regulation 882/2004, the official control activities should be conducted according to a previous programming based on risk analysis (Ramaswamy et al. 2007). The retrospective study of the results of the analysed samples is a fundamental tool for the identification of major risk foods and the planning of monitoring activities. The evaluation of the quality of the collected data may also allow for assessing the effectiveness of the controls previously implemented in the perspective of future improvements. The purpose of this study, therefore, is to explain critically the results of tests for the detection of *L. monocytogenes* performed by the IZSAM on food samples collected from 2000 to 2009 in the territory of Avezzano, Sulmona and Castel di Sangro (province of L'Aquila, Abruzzo).

Materials and methods

The samples were delivered to the Avezzano diagnostic section of IZSAM by the Public Veterinary Service (Avezzano, Sulmona and Castel di Sangro districts) and by the Food Hygiene and Nutrition Service (FINS) (only Avezzano district) of the Local Health Unit (LHU) of Avezzano-Sulmona-Castel di Sangro, as well as by the Carabinieri Health Protection Unit (NAS). Food samples were collected by means of official sampling at retail and at production and administration level as part of surveillance programs on food security.

The technique for the isolation of *L. monocytogenes* from food has been ISO 11290³ (Baek et al. 2000).

Results

The total number of samples tested for detection of *L. monocytogenes* is shown in Table I. The number of samples submitted is variable from year to

¹ European Commission (EC). 2004. Regulation (EC) N. 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules. *Off. J.* L 191, 28/05/2004.

² Centers for Disease Control and Prevention (CDC). 2012. Multistate outbreak of listeriosis linked to imported frescolina marte brand ricotta salata cheese (final update). www.cdc.gov/listeria/outbreaks/cheese-09-12/.

³ CEN ISO 11290-1:1996/Amd.1:2004. Microbiology of food and animal feeding stuffs - Horizontal method for the detection and enumeration of *Listeria monocytogenes* - Part 1: Detection method- Amendment 1: Modification of the isolation media and the haemolysis test and inclusion of precision.

year (Table I) and ranges from a minimum of 33 in 2008 (4.2%) to a maximum of 147 in 2004 (18.1%). In the period 2000-2002, the number of samples remains approximately the same, then it increases over the period 2003-2006 and subsequently decreases in 2007-2008, before returning to higher values in 2009 (Table I).

Table I. Total number of samples tested for *Listeria monocytogenes* between 2000 and 2009 in Avezzano, Sulmona and Castel di Sangro (province of L'Aquila, Abruzzo, Italy) by year.

Year	Total samples	%
2000	50	6.2
2001	62	7.6
2002	41	5.0
2003	102	12.6
2004	147	18.1
2005	121	14.9
2006	98	12.1
2007	48	5.9
2008	33	4.1
2009	110	13.5
Total	812	100.0

Figure 1 shows the percentage of samples in relation to the year and the sampling body. The Veterinary Service of Avezzano submitted 30.4% of the samples, Sulmona 61.1%, and Castel di Sangro 1.2%, NAS 2.6% and FINS 4.7%. Until 2004, the Veterinary Services of Avezzano and Sulmona have given mostly the same number of samples, from 2005 to 2009 the Veterinary Services of Sulmona has given the majority.

The examined samples were divided into categories related to those identified in the last EFSA Report on zoonoses (Farber and Peterkin 1991) (Table II). Even the food categories subject to sampling (Table II) are numerically different over the years. In fact, for dairy products, samples ranged from 7 in 2008 to 78 in 2009, for meat products the samples ranged from 5 in 2000 to 62 samples in 2004, while for fish products the samples ranged from 26 samples in 2003 to 2 in 2006 (no fish samples were considered during years 2007, 2008 and 2009). For other food matrices, samples ranged from 29 samples in 2004 to 2 in 2001, while there were no samples in 2000, 2003 and 2009. Dairy products were the majority (51.7%), followed by meat products (31.3%) and fish products (8.6%). The first 2 were received in greater numbers over the years, with meat products prevailing in a few cases

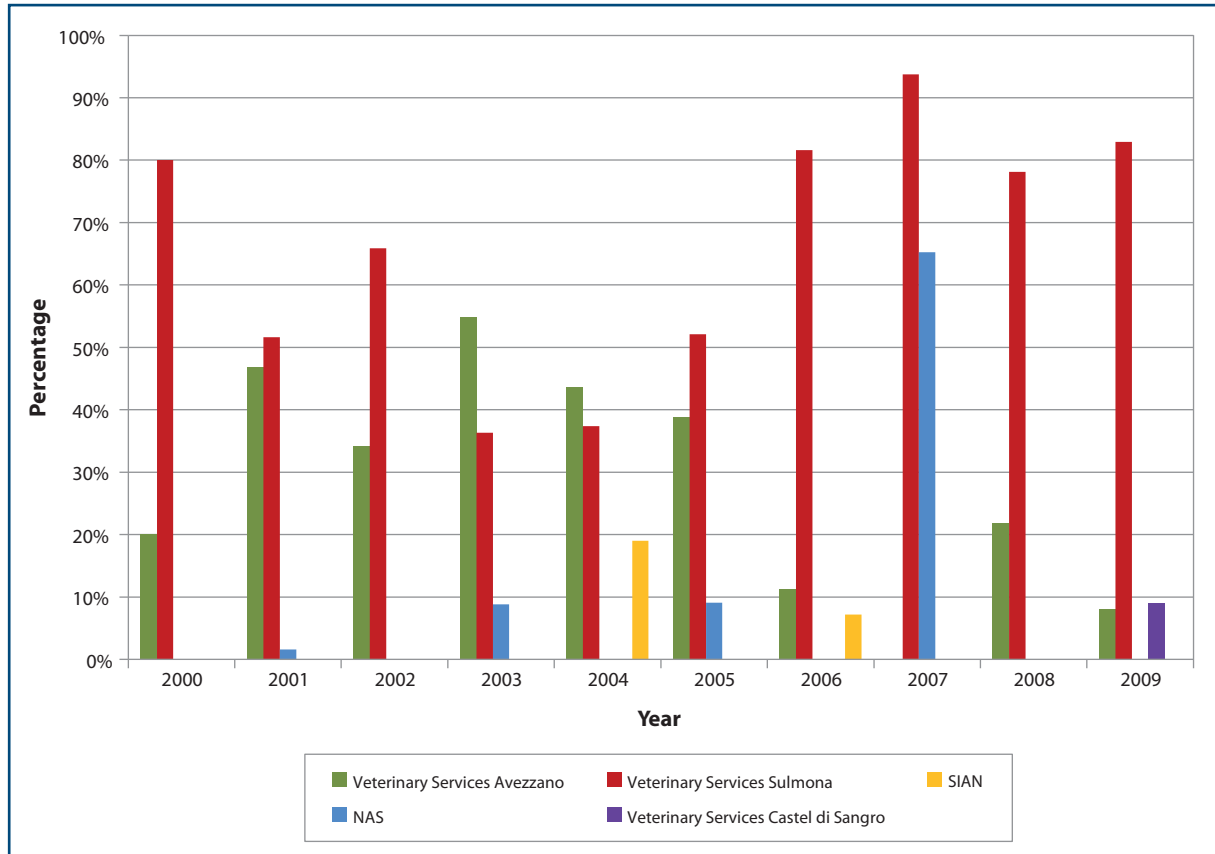


Figure 1. Percentage of samples collected from various body sampler in the period 2000-2009 in Avezzano, Sulmona and Castel di Sangro (province of L'Aquila, Abruzzo, Italy).

Table II. Number of samples tested for *Listeria monocytogenes* between 2000 and 2009 in Avezzano, Sulmona and Castel di Sangro (province of L'Aquila, Abruzzo, Italy) arranged by food group.

Food group	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	Total	%
Dairy products	40	32	11	35	46	74	67	30	7	78	420	51.7
Meat products	5	23	18	41	62	19	20	15	17	32	252	31.0
Fish products	5	5	5	26	10	17	2	0	0	0	70	8.6
Other food matrices	0	2	7	0	29	11	9	3	9	0	70	8.6
Total	50	62	41	102	147	121	98	48	33	110	812	100.0

Table III. Number of matrices examined tested for *Listeria monocytogenes* between 2000 and 2009 in Avezzano, Sulmona and Castel di Sangro (province of L'Aquila, Abruzzo, Italy) organised by group and type.

Category	Matrices	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	Total	%
Meat products	RTE meat products	0	6	8	16	37	5	8	2	4	17	103	12.7
	Non-RTE meat products	5	16	10	25	25	14	12	13	13	15	148	18.2
Fish products	RTE fish products	5	0	5	21	10	10	2	0	0	0	53	6.5
	Non-RTE fish products	0	5	0	5	0	7	0	0	0	0	17	2.1
Dairy	Cheese	40	33	10	25	36	61	56	30	1	40	332	40.9
	Heat-treated milk	0	0	1	5	0	7	1	0	0	22	36	4.4
	Bulk milk	0	0	0	0	0	0	0	0	5	16	21	2.6
	Other RTE products based on milk	0	0	0	5	10	6	10	0	1	0	32	3.9
Other	Other RTE foods	0	0	0	0	28	6	5	3	7	0	49	6.0
	Other non-RTE foods	0	2	7	0	1	5	4	0	2	0	21	2.6
Total		50	62	41	102	147	121	98	48	33	110	812	100.0

although, more often, the dairy ones proved to be in greater quantity. The majority of the fish products was collected in the period 2003 - 2005.

The submitted group of meat products included fresh meat from different animal species (pig, bovine, sheep, goat and poultry), cooked pig meat products (*porchetta*, *wurstel*, *mortadella*), fresh sausages and mature sausages (*salami*) and seasoned salted products (ham, loin and shoulder). The dairy products group included cattle and sheep cheeses, *mozzarella* cheese, ice cream, *ricotta*, milk and bulk milk, while the seafood products group included mainly salmon, fresh or smoked, as well as other species such as tuna, swordfish and flounder. The other food matrices consisted of various foods such as vegetables (salads, carrots, spinach and eggplant) and food preparation, including also prepared meals from canteens. Table III shows in detail the number of matrices examined over the period.

The monthly distribution of the samples received during the period under consideration is shown in Table IV. Most of the samples (38.3%) were submitted each year from March to May.

Percentages of isolation greater than 10% were observed in the years 2002, 2003, 2007 and 2009 (Figure 2). However, the percentages do not

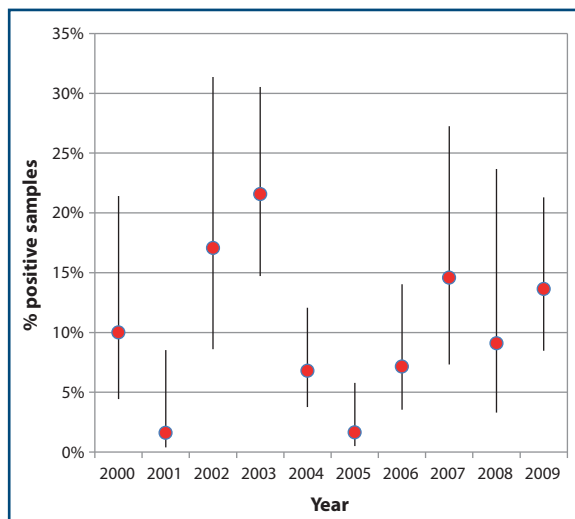
significantly differ from each other. Within the meat products group, the greater number of positive results has been recorded for fresh meat (4.0%) and sausages (*salame* and *sausage*) (14.3%); while, within fishery products, salmon (smoked or fresh) was the most contaminated product (40.0%). Only bovine bulk milk (0.5%) tested positive within dairy products. From 2007 to 2008, fish samples were no longer collected and therefore the higher percentage of isolation concerns meat products. For other food matrices, in 2002 a sample of bread skewers was found contaminated with *L. monocytogenes*.

Discussion

Listeriosis, a foodborne illness, is rather rare but serious diseases, whose economic and social impact is considered one of the highest among foodborne illnesses (Farber and Peterkin 1991). For this reason, a proper sampling conducted by the bodies in charge of surveillance [Veterinary Services, Food Hygiene and Nutrition Service (FINS), Carabinieri Health Protection Unit (NAS)] would be extremely valuable as it could help to clarify the prevalence and contamination levels of *L. monocytogenes* on most at risk food categories.

Table IV. Monthly distribution of the number of samples tested for *Listeria monocytogenes* between 2000 and 2009 in Avezzano, Sulmona and Castel di Sangro (province of L'Aquila, Abruzzo, Italy).

Months	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	Total	%
January	3	1	0	12	10	6	2	0	2	1	37	4.6
February	0	1	1	11	16	11	0	1	4	0	45	5.5
March	0	19	0	12	7	18	26	0	3	0	85	10.5
April	17	7	5	10	19	13	4	2	4	0	81	10.0
May	10	2	3	11	29	12	58	5	0	15	145	17.9
June	0	10	6	13	3	28	0	0	1	7	68	8.4
July	0	1	8	0	23	11	3	0	6	0	52	6.4
August	15	3	0	10	1	2	3	0	0	5	39	4.8
September	0	6	13	1	13	6	2	36	1	20	98	12.1
October	0	9	0	7	4	2	0	3	0	20	45	5.5
November	5	1	2	5	10	6	0	1	5	42	77	9.5
December	0	2	3	10	12	6	0	0	7	0	40	4.9
Total	50	62	41	102	147	121	98	48	33	110	812	100.0

**Figure 2.** Percentage of samples which resulted positive for *Listeria monocytogenes* between 2000 and 2009 in Avezzano, Sulmona and Castel di Sangro (province of L'Aquila, Abruzzo, Italy).

The data presented in this paper suggest the existence of deficiencies in planning preventative controls, especially for what concerns the provision that it should be based on risk analysis as required by Article 3 of the Reg. (CE) 882/2004.

In fact, the number of samples collected is variable from year to year (Table I) and also food categories considered are numerically different across the years (Table II). The number of matrices examined (grouped by RTE and non-RTE products) shows how the category of RTE products - whether they are derived from meat products, fish or dairy products - has not been taken sufficiently into account (Table III), while the most interesting results are those concerning the contamination prevalence of RTE products.

It is also worth noticing that the number of non-RTE samples is rather low (Table III), although a Ministerial Order of 1993⁴ expressly provides the sampling of non-RTE products as well.

The applied sampling plan, therefore, shows a lack of planning and goal setting. On the basis of historical and national reference, the food groups at risk of contamination by *L. monocytogenes* (i.e. those relevant for collecting samples) could be identified on yearly basis.

Referring to the distribution of the various matrices examined within the food categories in the considered period (Table III), we can see that their number changes from year to year and that some of them have been taken into account only for 1 or a few years, but always with a small number of samples. The definition of a sampling plan, instead, could allow the evaluation of the consumer exposure level, by focusing on the most at risk food or most consumed food, in order to define a criterion to identify the food matrices to be included in the plan.

Moreover, by considering the distribution of samples in different months from 2000 to 2009 (Table IV), it could be noted that the number of samples is not constant (sometimes even absent), in the course of the year. As a consequence, the food sampling do not meet the randomness that would be required to obtain usable data in order to highlight the possible presence of seasonality in food contamination by *L. monocytogenes*.

⁴ Italian Ministry of Health. 1993. Ordinanza of 7 December 1993. Limiti di *Listeria monocytogenes* in alcuni prodotti alimentari. *Off J*, **291**, 13.12.1993.

Therefore, it is unlikely that this type of data can provide comprehensive information on the risk factors. However, they could be used in order to assess the level of exposure of the consumer, taking into consideration the most at risk foods (or those most consumed) as a possible criterion for the identification of the matrices to be included in an organized sampling plan.

Regarding the percentage of positive samples for *L. monocytogenes* during the considered period, it was quite low and has never been linked to foodborne outbreaks. This can be explained by the fact that the disease occurs only as a result of contact with a high bacterial load⁵. Comparing the data obtained in the various categories with those obtained in the rest of Italy in similar studies, it may be noted that for meat products in the years 2001-2002 in Italy (Hudson *et al.* 1992) and in 2004 in Emilia Romagna⁶, the percentage of positive samples ranges from 3.6% to 4.6% for raw meats and processed meats respectively, to 16.0% for fresh red meat and 17.5% for fresh poultry meat, to 33.1% for minced meat and 26.9% in sausages. The matrices most represented are raw and processed meats (sausages like). The percentage of positive samples within fish category is 6.4% for fish and fish derivative products and 13% for prepared or preserved fish. The dairy product category presents low positive rates. The EFSA report on zoonosis of 2006 and 2007 reported positive rates for cattle and sheep cheeses ranging from 0.1% to 7.1% for 2006 and from 0.1% to 4.4% for 2007, while the Istituto Superiore di Sanità recorded a rate of 0.9% (Hudson *et al.* 1992). Also for other food matrices the percentages of positivity are low.

Moving on to examine some data relating to European countries such as Portugal (Miettinen *et al.* 2001), Spain⁷ (Vitas and Garcia-Jalon 2004.), Belgium

(Vazquez-Boland *et al.* 2001), Finland (Miettinen *et al.* 2001), meat products (especially raw meats and sausages) and fish products (fresh and smoked fish) categories result more positive than the others, with the exception of The Netherlands (Bergey's Manual of Systematic Bacteriology 2009) and Denmark (Notermans *et al.* 1998). The Netherlands and Denmark have percentages of positivity for *L. monocytogenes* in cheeses and dairy category of 10.0% and 18.9%, respectively. United Kingdom (McGowan *et al.* 1994), on the contrary, has a low percentage of positives for this food category and a high percentage for meat products. The data presented in studies concerning Chile (Cordano and Rocourt 2001), Japan (Inoue *et al.* 2000), India (Moharem Ahmed Saif *et al.* 2007), Ethiopia (Molla *et al.* 2004), New Zealand (Hudson *et al.* 1992), Australia (Ibrahim and Macrae 1991), Korea (Baek *et al.* 2000), tropics (Jeyasekaran *et al.* 1996), and the United States of America (Jinneman *et al.* 1999) show that the largest number of positivity is found in meat and fish products rather than in dairy products. Chile (Cordano and Rocourt 2001) and Ethiopia (Molla *et al.* 2004) recorded a positivity rate of 3.5% and 19.6%, respectively, for the matrix 'ice cream', while the positivity observed in Italy was very low (0.3%) (Gattuso *et al.* 2005). The comparison of the data shown in this work with those from studies conducted in Italy and in other states shows how the categories and matrices with higher percentages of positivity for *L. monocytogenes* are those that fall within the meat and fish products. In this case, the data collected from this study do not indicate a role of cheese as a vehicle of contamination in the areas sampled, in contrast to what reported in the national and international literature. It would therefore be necessary to promote an *ad hoc* sampling in the areas covered by this study to verify this aspect in more depth.

⁵ European Food Safety Authority (EFSA) 2009. The Community Summary Report on Trends and Sources of Zoonoses and Zoonotic Agents in the European Union in 2007. *EFSA Journal*, **223**, 1-313. www.efsa.europa.eu/de/scdocs/doc/223r.pdf.

⁶ Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna. 2005. Il controllo microbiologico sugli alimenti di origine animale in Emilia Romagna. Lettera di informazione, 62, settembre 2005. http://www.izsler.it/izs_bs/ftp/doc/controllo%20alimenti/micro04.pdf.

⁷ European Food Safety Authority (EFSA) 2007. The Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents, Antimicrobial Resistance and Foodborne Outbreaks in the European Union in 2006. *EFSA Journal*, **130**, 1-353. www.efsa.europa.eu/en/efsajournal/doc/130r.pdf.

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