Salmonella spp. in the feed chain in the Netherlands

Monitoring results of five years (2008 to 2012)

H. Yassin, P. Adamse and H.J. van der Fels-Klerx

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Summary

Salmonella spp. is an important food-borne pathogen in humans. In the Netherlands, monitoring *Salmonella* spp. in the feed and food chain has become an important issue since 1997. Monitoring results from different sectors, such as broiler meat and eggs, are analysed annually to determine the prevalence of S*almonella* spp.

The objective of this study was to analyse *Salmonella* spp. prevalence in feed materials in the Netherlands during the years 2008-2012. Data from the Dutch feed industry, stored in the GMP+ monitoring database, were provided by the Dutch Product Board Animal Feed for use in the current study. These data included results of, on average, 10080 compound feed and 9109 feed material samples per year. This high total number of samples reflects the intensive monitoring program in the Netherlands. The number of samples tested for *Salmonella* prevalence in feeds varied annually; for nearly all groups of feed materials and compound feeds, the number of samples stored in the database decreased significantly over the five study years. The decreasing number of samples may not reflect reality since – during the study years - results from samples with absence of *Salmonella* spp. were not collected consistently in the database.

Based on the available dataset, the highest Salmonella prevalence in compound feed for cattle was found during 2008 (0.5%) and the lowest during 2009 (0.1%). In compound feed for pigs, the highest Salmonella prevalence was seen during 2012 (0.5%) and the lowest during 2009 (0.2%). The highest Salmonella prevalence in compound feed for poultry was found in 2012 (0.8%) and the lowest in 2010 and 2011 (0.1% each). Compound feeds for laying hens was the main contributor to the high Salmonella prevalence in compound feed for poultry. In both compound feed for pigs and poultry, a significant increase in Salmonella prevalence was seen over the five study years.

In order to estimate the effect of not reporting samples without Salmonella spp. in the last years of the study, an estimate was made of the prevalence in each year, using the number of samples collected in the first year (2008). In this case, the Salmonella prevalence in compound feed for each of pigs, poultry in general, and laying hens decreased in 2009-2011, but increased again in 2012 to the same or higher prevalence as in 2008. In this scenario, the significant increase of Salmonella spp. in these three types of compound feeds during 2008-2012 was not significant anymore.

In feed materials, a fluctuation in Salmonella prevalence during the five years was seen with a high Salmonella prevalence during 2008 and 2012. In feed materials of land animal origin, the highest Salmonella prevalence was found in 2008 (4.9%) and the lowest during 2011 (1.1%). During the study years, a significant decreasing trend of Salmonella prevalence in this group of feed materials was seen. Greaves were the major source of Salmonella during several study years. In feed materials of marine animal origin, Salmonella was detected only during 2009 (0.4%) and 2010 (3.1%) with fishmeal as the main source. By-products of maize were the main source of Salmonella in feed materials of cereal grain origin. The highest Salmonella prevalence was found in 2010 (1.3%) and the lowest during 2008 (0.2%). In feed materials of oil seed origin, the highest Salmonella prevalence was found in 2012 (1.5%) and the lowest during 2011 (0.7%). The main sources of Salmonella during the five years were rapeseed, soya (bean), sunflower seed, and their byproducts. Salmonella was not found in premixes during the five years.

In 568 samples of compound feed and feed materials in Salmonella was present, the serovar was determined. In total 48 different Salmonella serovars were reported. The most commonly detected serovars in both feed types were: Senftenberg, Mbandaka, Agona, Livingstone and Cubana; all are considered non-pathogenic for human. Pathogenic serovars were found in 77 out of the 568 samples, mostly in compound feed for laying hens and feed materials of oil seed origin.

In conclusion, a high number of samples was collected each year for analysing the presence of Salmonella spp., and – general - this prevalence was low. The number of samples stored in the GMP+ monitoring database decreased over the five study years, probably because not all sample results with absence of *Salmonella* spp. were stored in the more recent years. Based on the available dataset, Salmonella prevalence seems to increase in compound feed for pigs and for poultry, with a highest prevalence of 0.8%, and to decrease in feed materials of land animal origin and feed material of plant origin other than cereals and oil seeds. The same is true when looking at the absolute numbers of Salmonella positives over the study years. In most cases, serovars present in the feed were not pathogenic to human.

It is recommended to focus sampling on compound feed and feed materials with the highest probability of being positive for *Salmonella* spp., being compound feeds for pigs and poultry and the feed materials of oil seeds origin, greaves, and maize (by-products). Sampling could be less intensive in the other groups of compound feeds and feed materials. Also, it is strongly recommended to store all monitoring results - not only Salmonella positive findings - in the database, such to enable drawing more reliable conclusions.

1 Introduction

Salmonella spp*.* is the most common foodborne pathogen worldwide. Over 2500 strains (serovars) of *Salmonella* are known*,* most of them rarely cause human disease (Grimont and Weill, 2007). However, certain strains such as *Salmonella* Enteritidis, Typhimurium, Hadar, Infantis, Virchow, Java, and Agona, may cause diseases in human.

Salmonella spp. are abundant in nature, being present everywhere in the environment. Salmonella is widely distributed in domestic and wild animals, including food producing animals such as poultry, pigs, cattle, as well as in pets, including cats and dogs, birds, and reptiles. Salmonella can also be found in many feeds. S*almonella* spp. can easily be transmitted between animals, within a herd or flock without being detected, and animals can become intermittent or persistent healthy carriers (Hugas and Beloeil, 2014). In the European Union, high priority is given to reduce food-borne diseases, amongst others for *Salmonellosis* in human (EC, 2002). The EFSA report on microbiological risk assessment in feedstuffs for food-producing animals (EFSA, 2008) revealed the existence of different sources for introducing *Salmonella* spp. into the animal production chain. However, in regions with a low prevalence, in which endemic infection is well controlled or absent, Salmonella contaminated feed is the major source for infections. The transmission of Salmonella from feed to animals consuming the feed, and to food products derived from the animals has been well documented (EFSA, 2008; Wales *et al.*, 2010; Jones, 2011; Li *et al.*, 2011). Though, according to the European Federation Fediol, transfer of Salmonella contamination from feed material, via the animal, to human is low (Fediol, 2014). The prevalence of Salmonella in poultry populations is considered the main risk factor for the presence of Salmonella in eggs and poultry meat (Hugas and Beloeil, 2014). Therefore, the control of *Salmonella* spp. in animal feeds is considered important in order to protect the transmission of Salmonella to the food chain.

In the Netherlands, control of *Salmonella* spp. in the poultry (feed) industry has become an important issue since 1997. In the European Union, a common legislative framework was established to ensure that domestic food of animal origin is free of *Salmonella* spp. (Directive 2003/99/EC and (EC) No 2160/2003). In accordance with the EC legislation (Directive 2003/99/EC), the prevalence of *Salmonella* spp. is monitored annually in the Dutch feed industry (EC, 2003). In 2002, a GMP$^+$ certification scheme has started to monitor *Salmonella* spp. in the animal feed industry. Annually, about 25,000 samples of feed materials are collected and tested for the presence of *Salmonella* spp. According to the Dutch Product Board Animal Feed (PDV), the prevalence of *Salmonella* spp. in poultry feed remained fairly constant at 0.3% between 2003-2007. Salmonella prevalence has considerably decreased in pig feed from 0.6% to 0.1% and in cattle feed from 0. 7% to 0.2% between 2003 and 2007 (PDV, 2008).

The objective of this study was to evaluate *Salmonella* spp. prevalence in animal feed in the Netherlands during the years 2008-2012. To this end, data collected by PDV during these years, stored in the GMP+ monitoring database, were used.

2 Materials and methods

Monitoring data of five years (2008 to 2012) of *Salmonella* spp. presence in different categories of animal feeds were used to evaluate the prevalence of *Salmonella* in animal feed in the Netherlands. Samples were collected by the Dutch feed industry according to the criterion set by the GMP+-certification scheme, "Monitoring salmonella in de diervoedersector 2002" and stored in the GMP+ monitoring database. Subsequently samples were tested for *Salmonella* spp. at a well-recognized GMP^++B10 certified laboratory. Usually *Salmonella* spp. positive samples are sent to the Institute for Public Health and the Environment (RIVM) or another GMP^+ + B10 certified laboratory for serotyping (PDV, 2008). Afterwards, serovars would be serotyped according to the procedures written in ISO/TR 6579-3:2014 (Anonymous, 2014).

Data included various compound feeds, feed materials and premixes. On average 19,414 samples of compound feeds, feed materials and premixes were analysed annually. Data of compound feed included that of cattle, pigs, poultry (laying hens, breeders, broilers, ducks and turkeys), horses, and other animals (pets and small ruminants). Data of feed materials included that of feed material of animal origin, feed material of cereal grain origin, feed material of oil seed origin, feed material of other plant origin, and premixes. Data of feed material of animal origin included that of animals of land origin and of marine origin. Data of feed material of cereal grain origin included that of cereals (such as barley, wheat, maize, oats) as well as their byproducts. Data of feed material of oil seed origin included that of oil seeds (such as groundnut, rapeseed, palm kernel, soya (bean), cotton seeds, sunflower seeds, linseed) as well as their byproducts. Data of feed material of other plant origin included that of forages, silage, fruit, legumes, tubers, roots, etc.

Data were analysed in Excel. The prevalence of *Salmonella* spp. (SP) in different feed categories was calculated from the number of positive samples reported in each feed category divided by the total number of samples tested for that specific feed category. Positive samples are those samples which express *Salmonella* spp. during the test, i.e., 1 colony forming unit (CFU) per 25 gram of sample material tested. For each feed category, a linear trend line was fitted to the annual data for both the number of samples and SP. The R^2 of both the fitted trend lines (number of samples, SP) was calculated. An R^2 of 0.30 or higher indicates a significant trend over the five study years.

3 Results

Annually, between about 10,000 and 27,000 samples of feeds were tested for *Salmonella* spp., with an overall average of 19.414 samples per year. Table 1 shows the total number of feed samples tested for *Salmonella* spp. during 2008-2012.

Table 1
Number of samples tested for Salmonella spp. in compound feed and feed materials during 2008-2012 in the Netherlands.

	2008	2009	2010	2011	2012	Average
Feed categories						
Compound feed	11,924	14,105	8520	9297	6555	10,080
Feed materials	15,180	10,616	7253	8744	3750	9109
Premixes	168	285	164	359	148	225
Total	**27,272**	**25,006**	**15,937**	**18,400**	**10,453**	**19,414**

3.1 Compound feeds

In total 11,924, 14,105, 8,520, 9,297, and 6,555 compound feed samples were tested during 2008, 2009, 2010, 2011, and 2012, respectively. The number of samples decreased significantly over the study years (R^2=0.69). In these five years, respectively, 38 (0.32%), 18 (0.13%), 14 (0.16%), 16 (0.18%), and 37 (0.56%) samples were found Salmonella positive. The overall Salmonella prevalence in 2012 was attributed to the high Salmonella prevalence in compound feed for laying hens (1.9%), followed by that of pigs (0.5%). Figure 1 shows the annual Salmonella prevalence in the total number of compound feed samples tested during the five years. There was no significant (decreasing or increasing) trend in the prevalence of Salmonella during the five years (R^2 =0.26).

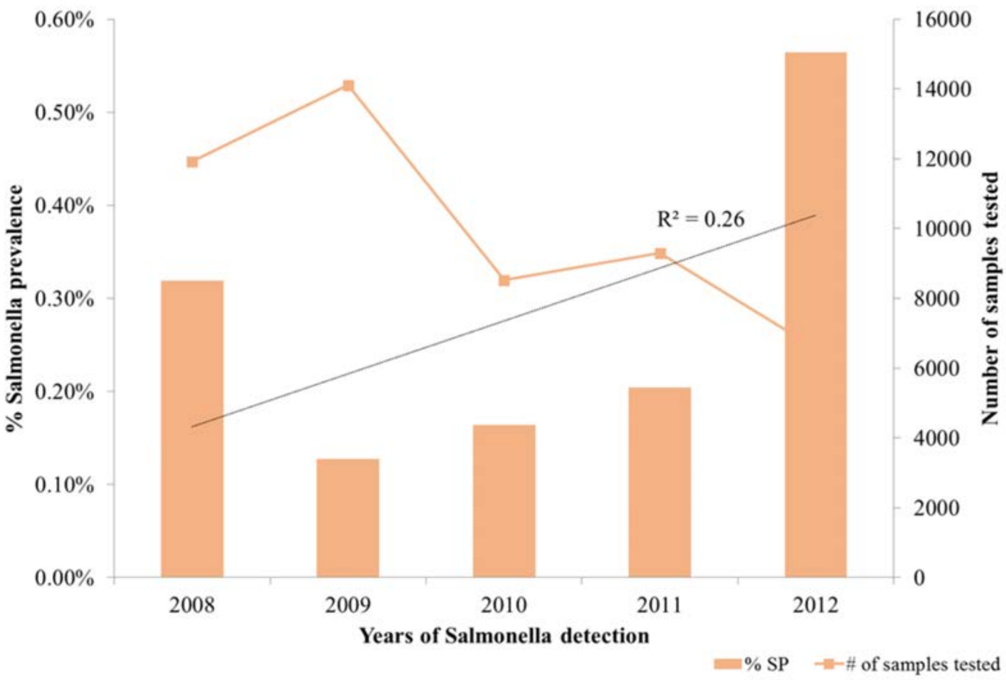

Figure 1 *Annual Salmonella spp. prevalence (SP) in all compound feed samples tested during 2008-2012.*

During the five years, 27353, 8783, 11547 samples of, respectively, compound feed for poultry, cattle and pigs were tested. In total, 64 (0.23%), 24 (0.27%) and 33 (0.29%) of the total samples of compound feed for, respectively, poultry, cattle and pigs were found Salmonella positive. Figure 2 shows the Salmonella prevalence for the compound feeds per animal category during the five years. Of the different groups of compound feed for poultry, Salmonella was not found in compound feed for ducks and turkeys, and Salmonella was not present in compound feed for broilers during three years (2010, 2011, and 2012). Results of Salmonella prevalence in compound feed during the years 2008 to 2012 are summarized in Table 2, and presented in detail in the following sections.

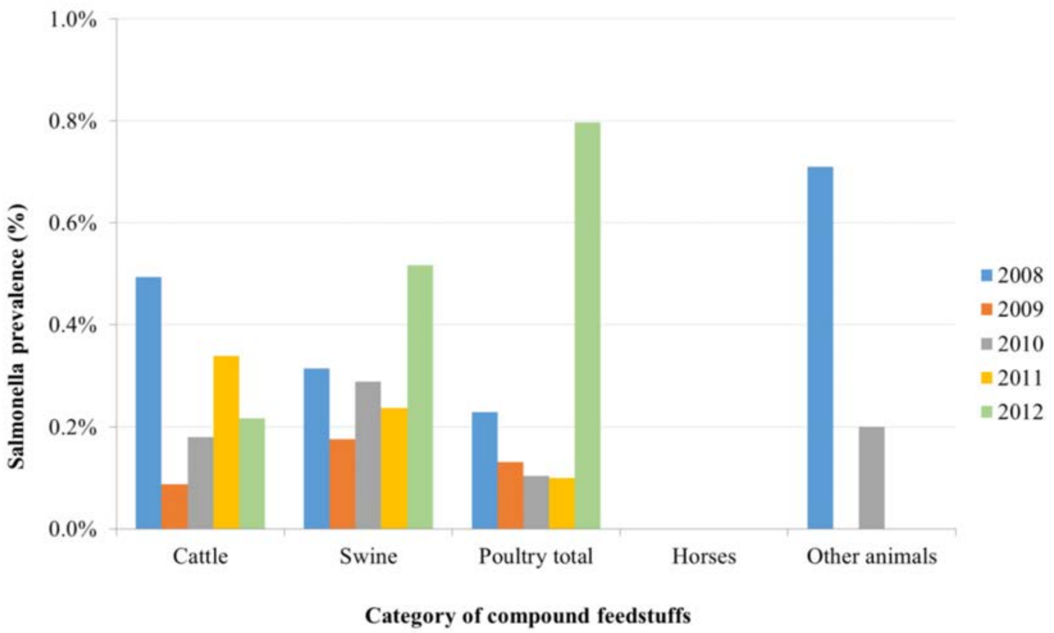

Figure 2 *Annual Salmonella spp. prevalence in compound feed for different animal categories during 2008-2012.*

3.1.1 Compound feed for cattle

In total, 2229, 2287, 1111, 1770, and 1386 samples of compound feed for cattle were analyzed during the years 2008, 2009, 2010, 2011, and 2012, respectively (Table 2). For those years, the number of *Salmonella* spp. positive samples were, respectively, 11 (0.49%), 2 (0.09%), 2 (0.18%), 6 (0.34%), and 3 (0.22%). The highest Salmonella prevalence was found in 2008 and the lowest in 2009. Figure 3 shows the Salmonella prevalence during the five years. There was no (decreasing or increasing) trend in the prevalence of Salmonella during the five years (R^2 =0.09).

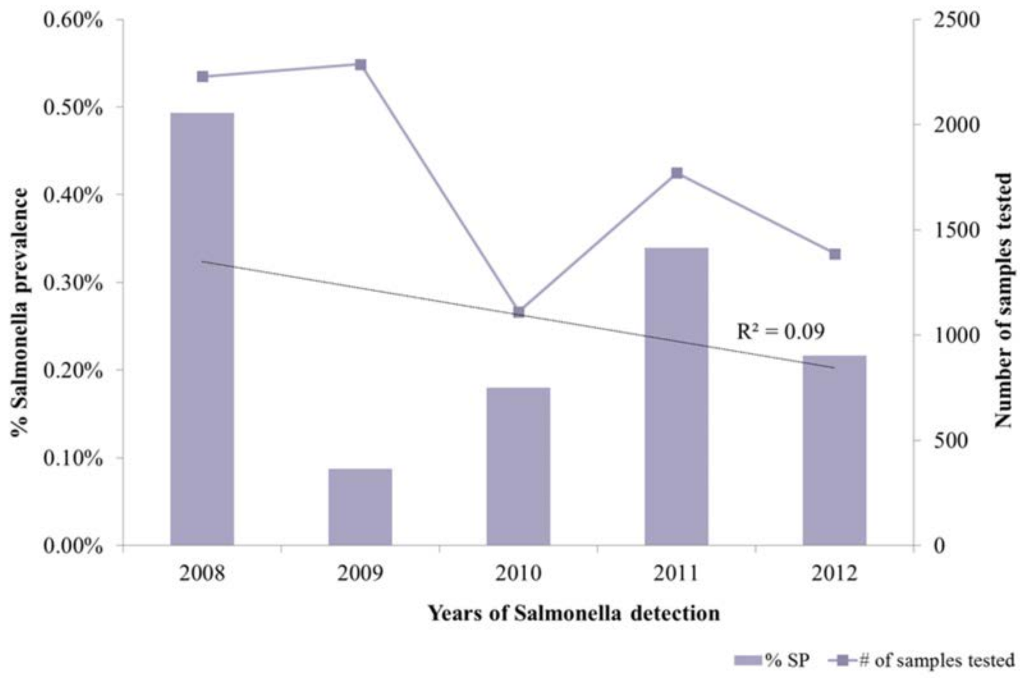

Figure 3 *Annual Salmonella spp. prevalence (SP) in compound feed for cattle during 2008-2012.*

3.1.2 Compound feed for pigs

In total, 2543, 2842, 2080, 2531, and 1551 samples of compound feed for pigs were analysed during the years 2008, 2009, 2010, 2011, and 2012, respectively (Table 2). The number of Salmonella positive samples for those five years were, respectively, 8 (0.31%), 5 (0.18%), 6 (0.29%), 6 (0.24%), and 8 (0.52%). The highest Salmonella prevalence in compound feed for pigs was found in 2012, and the lowest in 2009. Figure 4 shows the Salmonella prevalence during the five years. The prevalence of Salmonella increased significantly over the study period (R^2= 0.33).

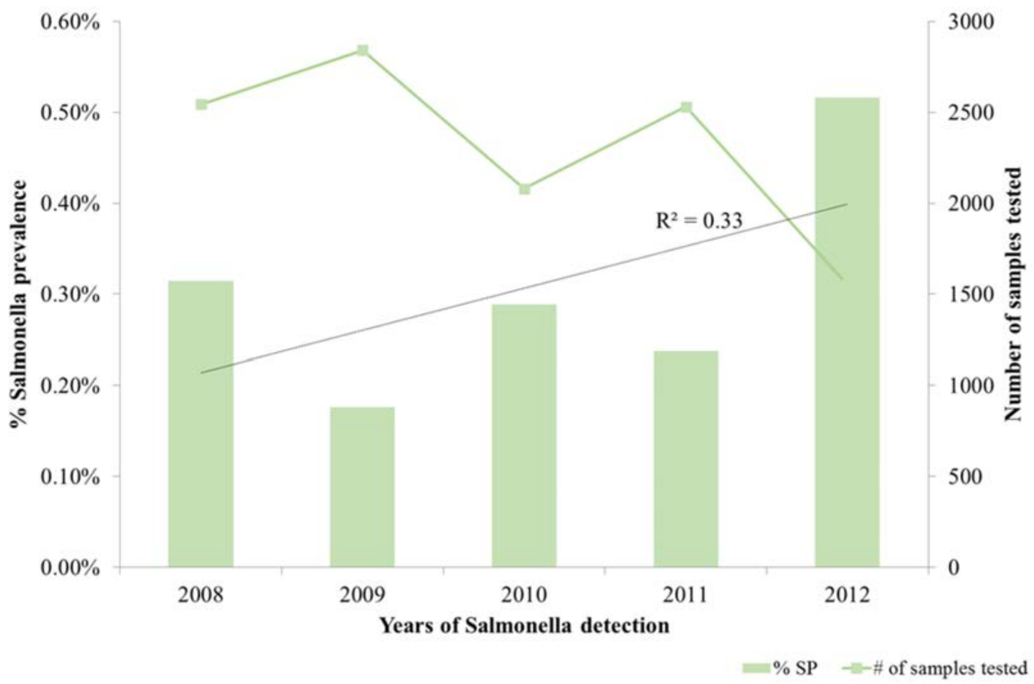

Figure 4 *Annual Salmonella spp. prevalence (SP) in compound feed for pigs during 2008-2012.*

3.1.3 Compound feed for poultry

Compound feed for poultry included compound feed for breeders, laying hens, broilers, ducks, turkeys and other non-specified poultry. In total, 6547, 8411, 4797, 4333, and 3265 samples of compound feed for poultry were tested during the years 2008, 2009, 2010, 2011, and 2012, respectively (Table 2). For those five years, the number of Salmonella positive samples were, respectively, 15 (0.23%), 11 (0.13%), 5 (0.10%), 4 (0.10%), and 26 (0.80%). The highest Salmonella prevalence was thus found in 2012, and the lowest in 2010 and 2011. Figure 5 shows the Salmonella prevalence in compound feed for poultry during the five years. The increasing trend over the five years was significant (R^2=0.34).

The Salmonella prevalence in compound feed for different poultry groups during 2008-2012 is shown in Figure 6, and outlined below.

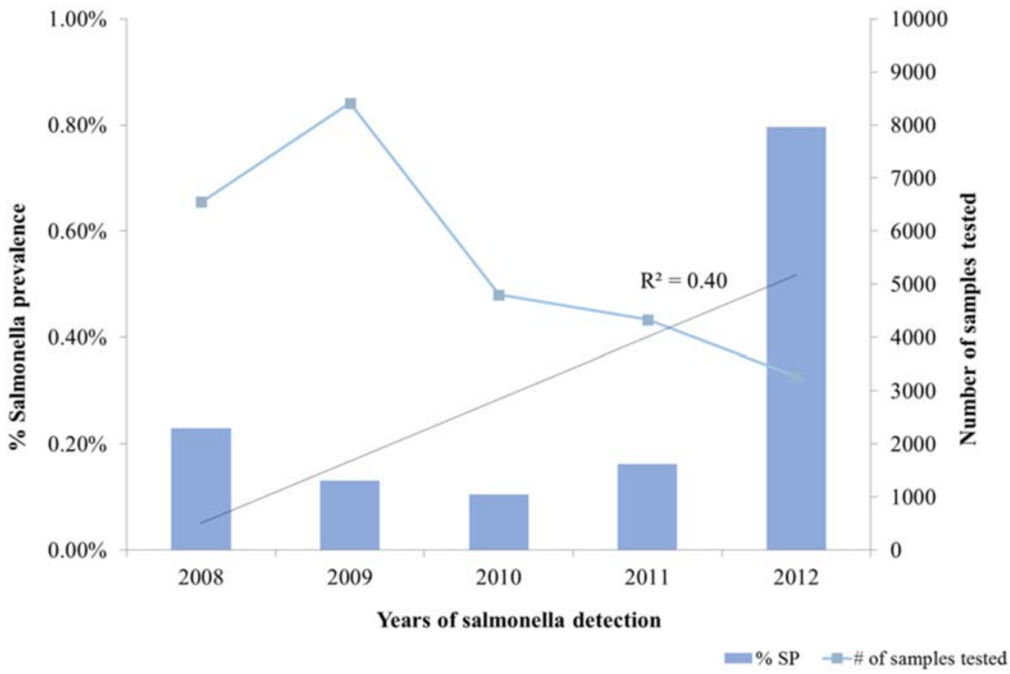

Figure 5 *Annual Salmonella spp. prevalence (SP) in compound feed for poultry during 2008-2012.*

3.1.3.1 Compound feed for breeders

In total, 1,581, 2,165, 618, 515, and 814 samples of compound feed for breeders were tested during the years 2008, 2009, 2010, 2011, and 2012, respectively (Table 2). Of the total numbers of samples tested, respectively, 2 (0.13%), 0, 0, 1 (0.19%), and 2 (0.25%) samples were Salmonella positive during those five years. For compound feed for breeders, the highest Salmonella prevalence was found in 2012, and the lowest in 2009 and 2010 (Figure 6).

3.1.3.2 Compound feed for laying hens

In total, 3,007, 3,692, 2,196, 1,839, and 1,300 samples of compound feed for laying hens were tested during the years 2008, 2009, 2010, 2011, and 2012, respectively (Table 2). For those five years, the numbers of Salmonella positive samples were, respectively, 12 (0.40%), 10 (0.27%), 5 (0.23%), 6 (0.33%), and 24 (1.85%). For compound feed for laying hens, the highest Salmonella prevalence was found in 2012, and the lowest in 2010 (Figure 6).

3.1.3.3 Compound feed for broilers

In total, 1,840, 1,828, 1,334, 1,434, and 929 samples of compound feed for broilers were tested during the years 2008, 2009, 2010, 2011, and 2012, respectively (Table 2). Of the total samples tested, one (0.05%) sample was Salmonella positive in each of 2008 and 2009, and *Salmonella* was not detected during the remaining three years (Figure 6).

3.1.3.4 Compound feed for ducks

In total, 35, 66, 67, and 20 samples of compound feed for ducks were tested during the years 2009, 2010, 2011, and 2012, respectively (Table 2). In 2008, no samples were tested for Salmonella. Salmonella was not detected in compound feed for ducks (Figure 6).

3.1.3.5 Compound feed for turkeys

In total, 423, 213, 254, and 76 samples of compound feed for different groups of turkeys were tested during the years 2009, 2010, 2011, and 2012 respectively (Table 2). None of the samples showed presence of Salmonella (Figure 6). No samples were tested in 2008.

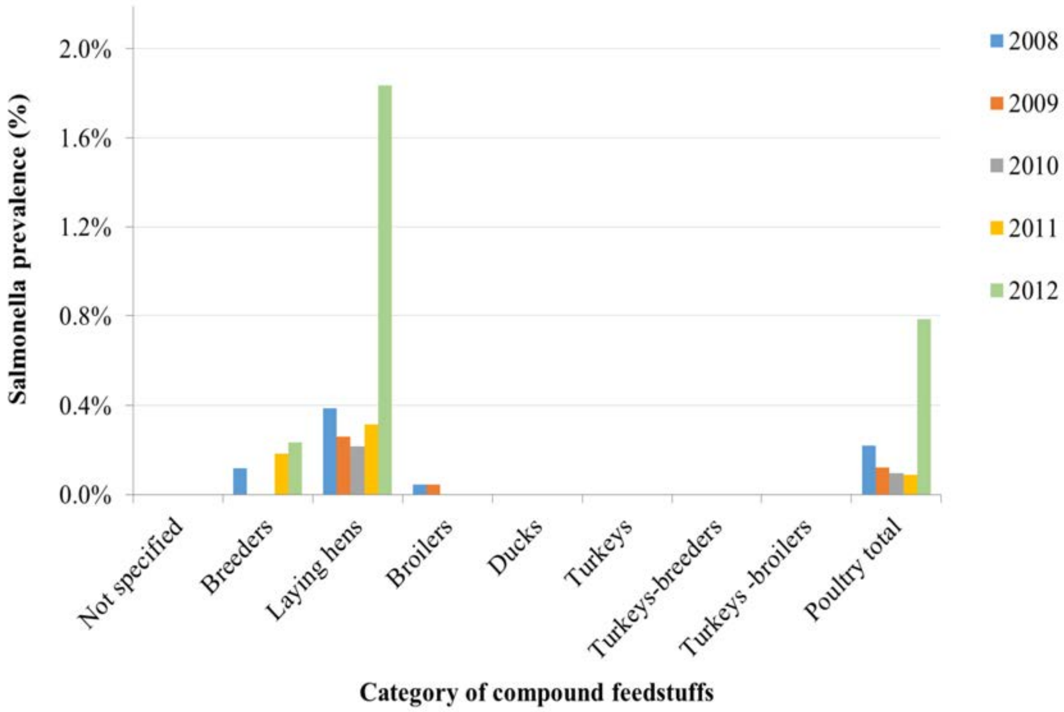

Figure 6 *Salmonella spp. prevalence in compound feed for different groups of poultry during 2008-2012.*

3.1.4 Compound feed for horses, pets and other ruminants

In total, 41, 52, 34, 47, and 64 samples of compound feed for horses were tested during the years 2008, 2009, 2010, 2011, and 2012, respectively (Table 2). Salmonella was not found during those five years.

For pets such as, dogs, cats and rabbits, 30, 60, 395, 551, and 211 samples were tested for the years 2008 to 2012, respectively (Table 2). In 2010, one sample (0.25%) was Salmonella positive. Salmonella was not present in the other four years.

For other ruminants such as, goats and sheep, respectively, 534, 453,103, 65, and 78 samples were tested in total during those five years (Table 2). In 2008, four samples (0.71%) were found Salmonella positive. Salmonella was not present in the other four years (Table 2).

Table 2

Results for Salmonella prevalence (SP) in compound feed in the Netherlands during 2008-2012.

Category of compound feedstuffs	2008			2009			2010			2011			2012		
	# tested	# pos	SP (%)	# tested	# pos	SP (%)	# tested	# pos	SP (%)	# tested	# pos	SP (%)	# tested	# pos	SP (%)
Cattle	2229	11	0.49	2287	2	0.09	1111	2	0.18	1770	6	0.34	1386	3	0.22
Pigs	2543	8	0.31	2842	5	0.18	2080	6	0.29	2531	6	0.24	1551	8	0.52
Poultry															
Not specified	119	0	0.00	268	0	0.00	370	0	0.00	224	0	0.00	126	0	0.00
Breeders	1581	2	0.13	2165	0	0.00	618	0	0.00	515	1	0.19	814	2	0.25
Laying hens	3007	12	0.40	3692	10	0.27	2196	5	0.23	1839	6	0.33	1300	24	1.85
Broilers	1840	1	0.05	1828	1	0.05	1334	0	0.00	1434	0	0.00	929	0	0.00
Ducks				35	0	0.00	66	0	0.00	67	0	0.00	20	0	0.00
Turkeys-other				342	0	0.00	159	0	0.00	217	0	0.00	27	0	0.00
Turkeys-breeders										10	0	0.00	18	0	
Turkeys -broilers				81	0	0.00	54	0	0.00	27	0	0.00	31	0	0.00
Poultry total	**6547**	**15**	**0.23**	**8411**	**11**	**0.13**	**4797**	**5**	**0.10**	**4333**	**4**	**0.10**	**3265**	**26**	**0.80**
Horses	41	0	0.00	52	0	0.00	34	0	0.00	47	0	0.00	64	0	0.00
Other animals	564	4	0.71	513	0	0.00	498	1	0.20	616	0	0.00	289	0	0.00
Total all animals	**11924**	**38**	**0.32**	**14105**	**18**	**0.13**	**8520**	**14**	**0.16**	**9297**	**16**	**0.18**	**6555**	**37**	**0.56**

3.2 Feed materials

In total, 15,180, 10,616, 7253, 8744, and 3750 samples of feed materials were tested during 2008, 2009, 2010, 2011, and 2012, respectively. The number of samples tested decreased significantly over the study years (R^2=0.85). In the five years, respectively, 172 (1.1%), 73 (0.7%), 75 (1.0%), 66 (0.8%), and 38 (1.0%) samples were found Salmonella positive. There was no significant decreasing or increasing trend in Salmonella prevalence in all feed materials during the study period. Salmonella prevalence was highest in 2008 and lowest in 2009. In 2008, the overall Salmonella prevalence was attributed to the high Salmonella prevalence in feed materials of land animal origin, especially greaves, as well as in feed materials of oil seed origin. Figure 7 presents the annual Salmonella prevalence in the total number of feed material samples tested during the five years.

During the five years, 2460, 687, 935, 7814, 31699, 1868, and 1124 samples of milk and milk products; feed materials of land animal origin; feed materials of marine animal origin; feed materials of cereal grain origin; feed materials of oil seed origin; feed materials of other plant origin; and premixes were tested, respectively. Out of the total group of feed materials, the highest number of samples tested were from feed materials of oil seed origin. The highest prevalence of Salmonella was found in feed materials of land animal origin (3.1%). The second highest prevalence of Salmonella was found in feed materials of oil seed origin (1.1%). Figure 8 shows the annual Salmonella prevalence during the five years, in the different categories of feed materials. Results of Salmonella prevalence in feed materials in the period 2008 - 2012 are also shown in Table 3, and presented in detail in the following sections. There was no (decreasing or increasing) trend in the prevalence of Salmonella during the five years (R^2 =0.02).

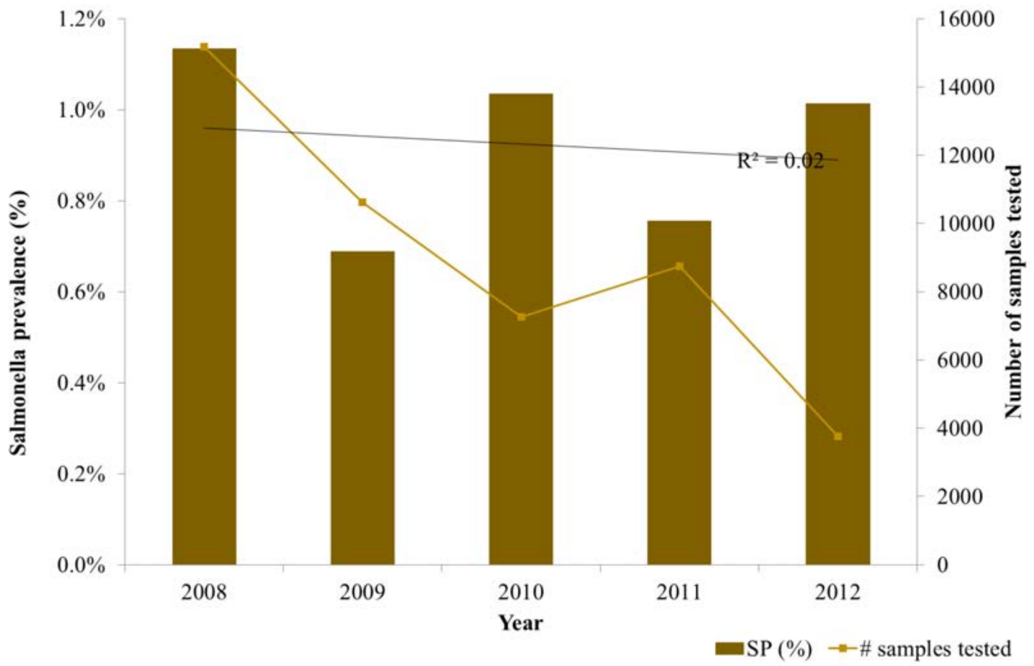

Figure 7 *Overall annual Salmonella spp. prevalence (SP) in all feed materials tested during 2008-2012.*

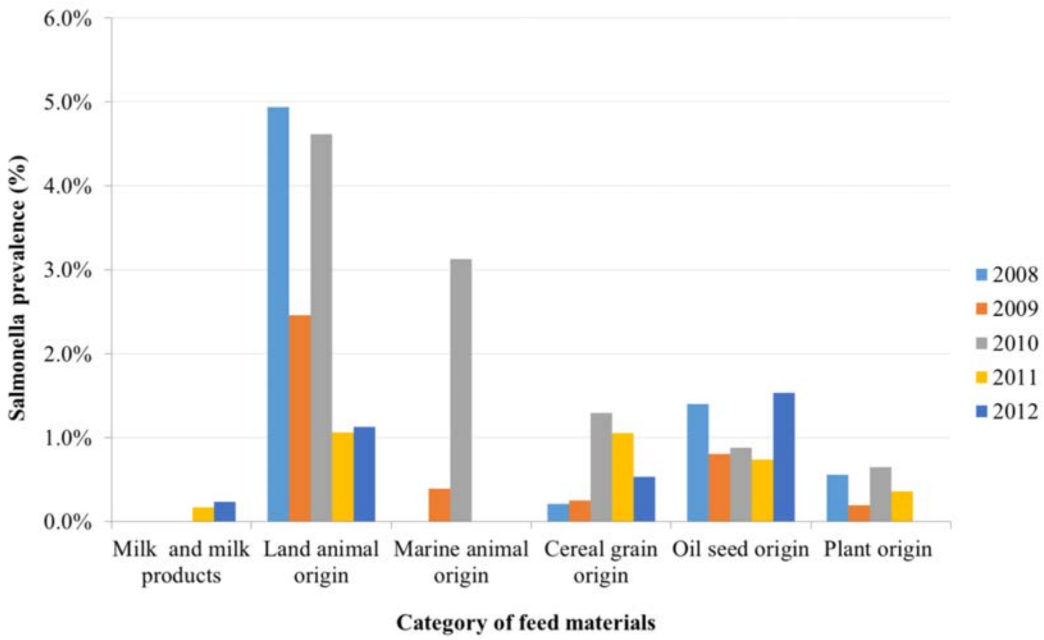

Figure 8 *Annual Salmonella spp. prevalence in all sources of feed materials during 2008-2012.*

3.2.1 Milk and milk products

In total, 567, 547, 319, 600, and 427 samples of milk and milk products were analysed during the years 2008, 2009, 2010, 2011, and 2012, respectively (Table 3). Of the total samples tested, Salmonella was not found during the years 2008, 2009 and 2010. One sample (0.2%) was Salmonella positive in each of 2011 and 2012. The sources of Salmonella in those years were cheese products and whey protein concentrate, respectively.

3.2.2 Feed materials of land animal origin

In total, 162, 163, 130, 94, and 177 samples of feed materials of land animal origin were analysed during the years 2008, 2009, 2010, 2011, and 2012, respectively (Table 3). For those five years, the number of Salmonella positive samples were, respectively, 8 (4.9%), 4 (2.5%), 6 (4.6%), 1 (1.1%) and 2 (1.1%). The highest Salmonella prevalence was thus found in 2008 and the lowest in 2010 and 2011. The decrease in Salmonella prevalence over the five years was significant (R^2=0.59). Greaves were the main source of Salmonella. Figure 9 shows the Salmonella prevalence in different feed sources of land animal origin during the five year period. Figure 10 shows the annual Salmonella prevalence in feed materials of land animal origin.

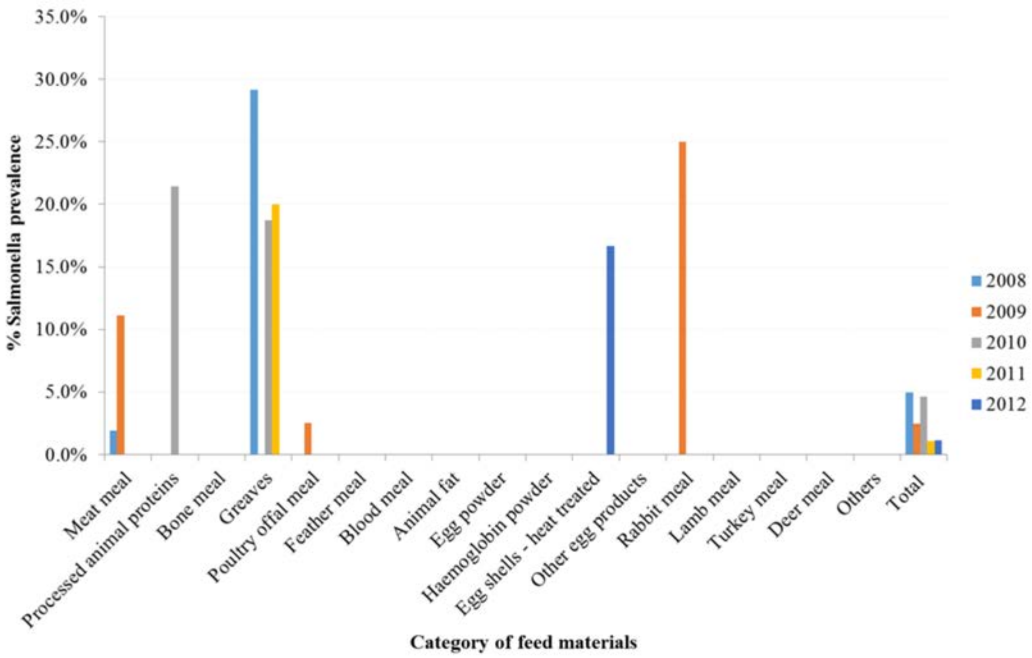

Figure 9 *Salmonella spp. prevalence in feed materials of land animal origin during 2008-2012.*

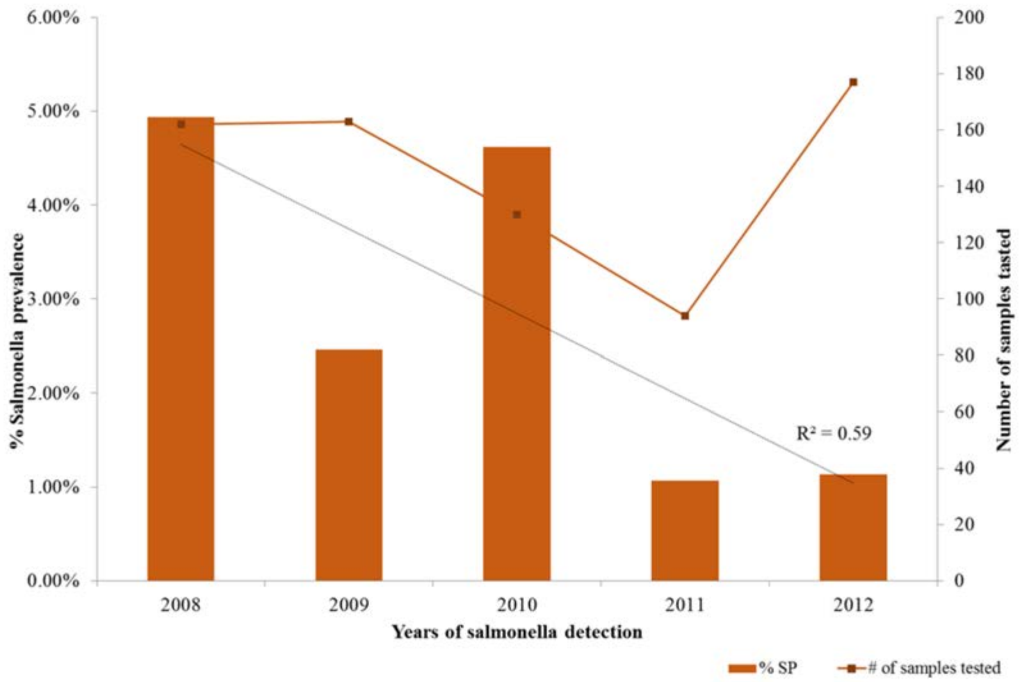

Figure 10 *Annual Salmonella spp. prevalence (SP) in feed materials of land animal origin during 2008-2012.*

3.2.3 Feed materials of marine animal origin

In total, 344, 256, 192, 138, and 43 samples of feed materials of marine animal origin were analysed during the years 2008, 2009, 2010, 2011, and 2012, respectively (Table 3). Salmonella was not found during the years 2008, 2011, and 2012. In 2009 and 2010, one sample (0.4%) and six samples (3.1%) were Salmonella positive, respectively. The highest Salmonella prevalence was found in 2010. The main source of Salmonella was fish meal.

3.2.4 Feed materials of cereal grain origin

In total, 2352, 1198, 1233, 2086, and 945 samples of feed materials of cereal grain origin were analysed during the years 2008, 2009, 2010, 2011, and 2012, respectively (Table 3). The number of Salmonella positive samples for those five years were, respectively, 5 (0.2%), 3 (0.3%), 16 (1.3%), 22 (1.1%), and 5 (0.5%). The highest Salmonella prevalence was thus found in 2010 and the lowest in 2008. The main source of Salmonella were by-products of maize. Figure 11 shows the prevalence of Salmonella in different categories of feed materials of cereal grain origin. The annual Salmonella prevalence in feed materials of cereal grain origin is shown in Figure 12. There was no (decreasing or increasing) trend in the prevalence of Salmonella during the five years (R^2 =0.22).

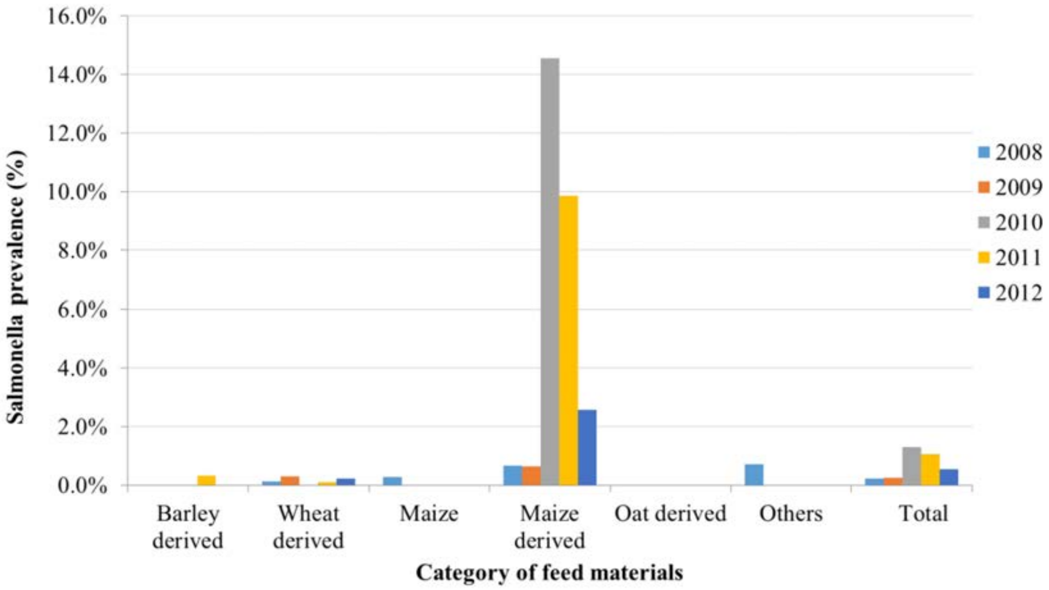

Figure 11 *Salmonella spp. prevalence in feed materials of cereal grain origin during 2008-2012.*

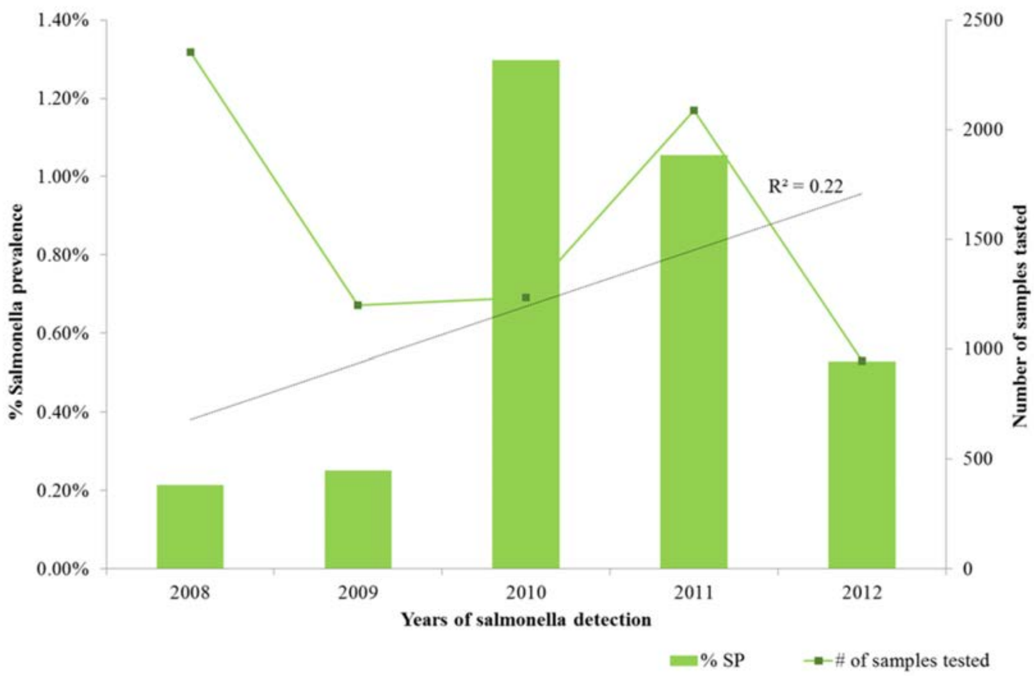

Figure 12 *Annual Salmonella spp. prevalence (SP) in feed materials of cereal grain origin during 2008-2012.*

3.2.5 Feed materials of oil seed origin

In total, 11037, 7939, 5225, 5546, and 1955 samples of feed materials of oil seed origin were analysed during the years 2008, 2009, 2010, 2011, and 2012, respectively (Table 3). For those five years, the number of Salmonella positive samples were, respectively, 155 (1.4%), 64 (0.8%), 46 (0.9%), 41 (0.7%), and 30 (1.5%). The number of samples analyzed decreased significantly (R^2=0.92) over the study years. No significant decrease or increase of the prevalence of Salmonella over the years was seen (Figure 14, R^2=0.01). The highest Salmonella prevalence was found in 2012 and the lowest in 2011. The main sources of Salmonella during the five years were rapeseed, soya (bean), sunflower seed and their by-products. Figure 13 shows the prevalence of Salmonella in different categories of feeds from oil seed origin. Annual Salmonella prevalence in feed materials of oil seed origin is shown in Figure 14.

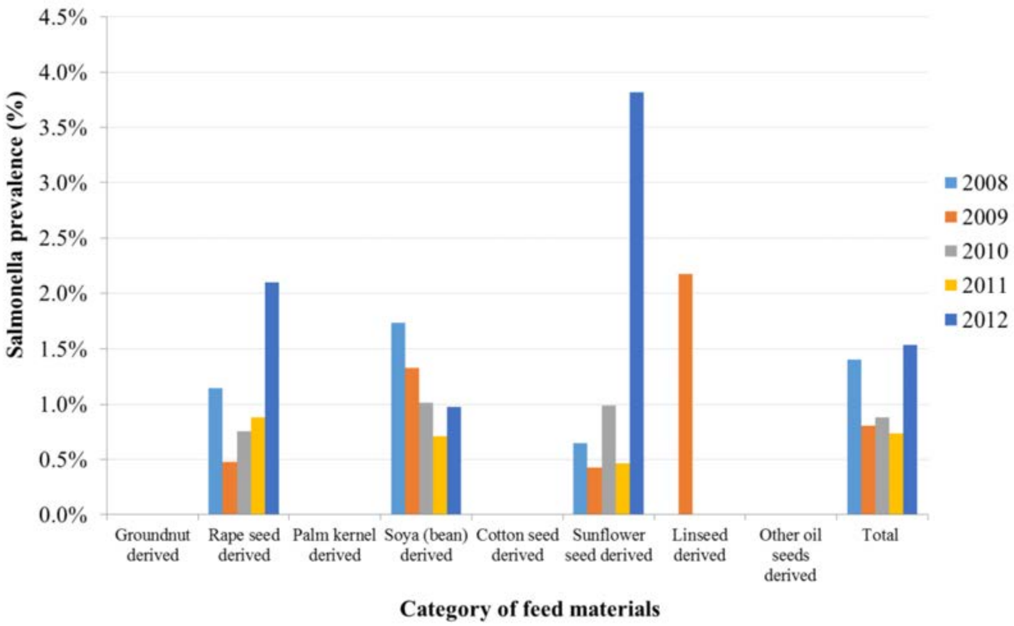

Figure 13 *Salmonella spp. prevalence in feed materials of oil seed origin during 2008-2012.*

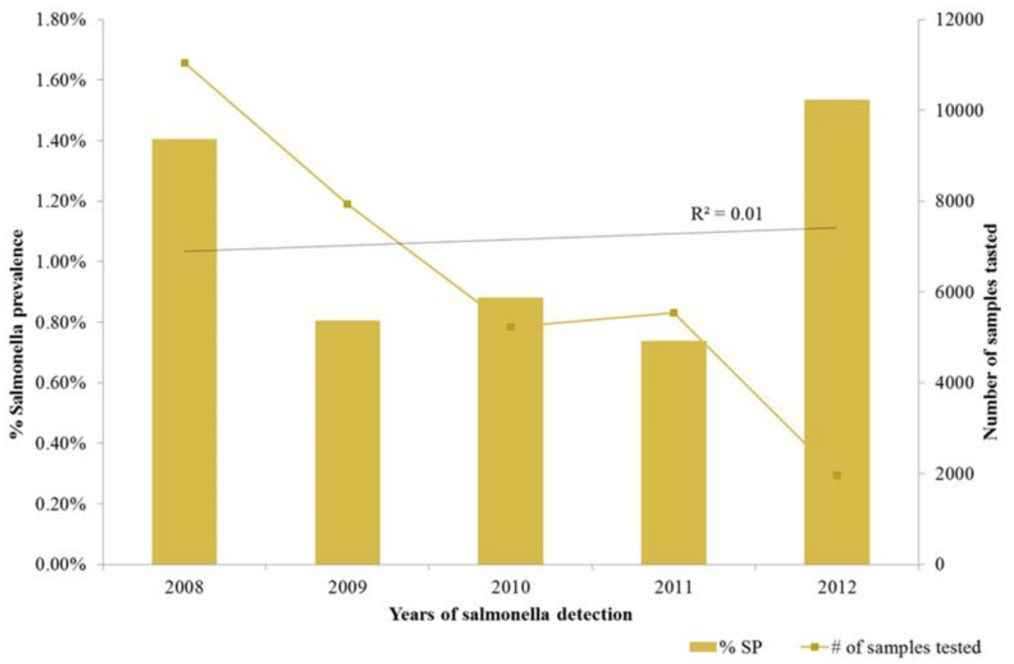

Figure 14 *Annual Salmonella spp. prevalence (SP) in feed materials of oil seed origin during 2008-2012.*

3.2.6 Feed materials of plant origin other than cereals and oil seeds

In total, 718, 513, 154, 280, and 203 samples of feed materials of plant origin other than cereals and oil seeds were analysed during the years 2008, 2009, 2010, 2011, and 2012, respectively (Table 3). The numbers of Salmonella positive samples for those five years were, respectively, 4 (0.6%), 1 (0.2%), 1 (0.6%), 1 (0.4%), and 0 (0%). Both the number of samples analysed (R^2=0.71) and the Salmonella prevalence (R^2=0.32) decreased significantly over the study years. The highest Salmonella prevalence was found in 2008 and 2010, and the lowest in 2012.. The annual Salmonella prevalence in feed materials of plant origin other than cereals and oil seeds is shown in Figure 15.

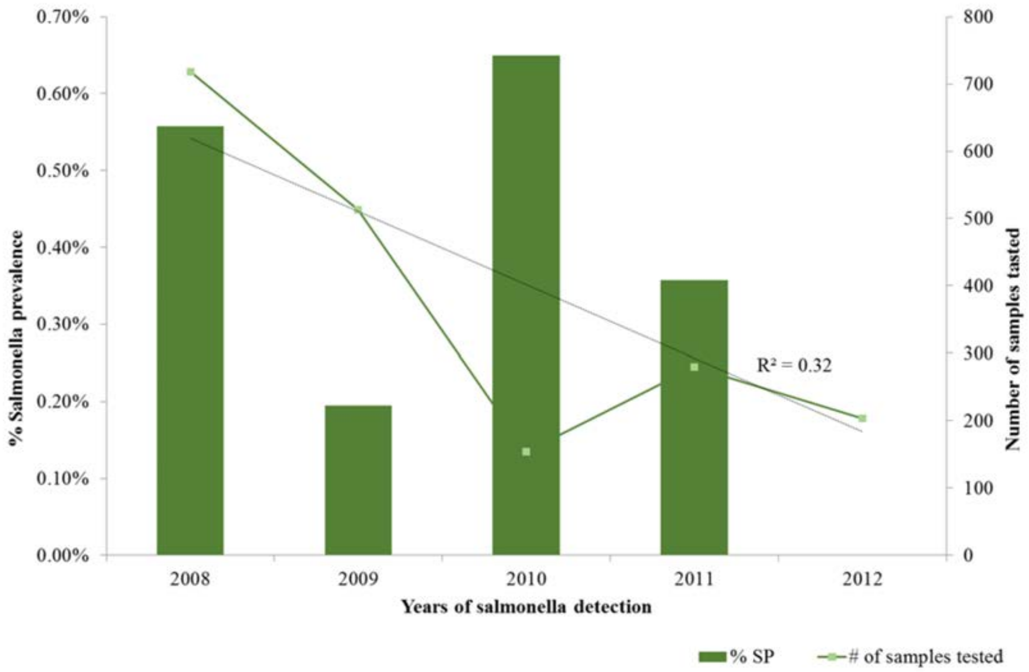

Figure 15 *Annual Salmonella spp. prevalence (SP) in feed materials of plant origin other than cereals and oil seeds during 2008-2012.*

3.3 Premixes

In total 168, 285, 164, 359 and 148 samples of premixes were analysed during the years 2008, 2009, 2010, 2011 and 2012, respectively. Salmonella was not found in any of these samples tested.

Table 3

Salmonella spp. prevalence (SP) in feed materials in the Netherlands during 2008-2012.

	2008			2009			2010			2011			2012		
Category of feed materials	**# tested**	**# pos**	**SP (%)**	**# tested**	**# pos**	**SP (%)**	**# tested**	**# pos**	**SP (%)**	**# tested**	**# pos**	**SP (%)**	**# tested**	**# pos**	**SP (%)**
Milk and milk products	567	0	0.0	547	0	0.0	319	0	0.0	600	1	0.2	427	1	0.2
Feed material of land animal origin															
Meat meal	53	1	1.9	18	2	11.1	47	0	0.0	4	0	0.0	24	0	0.0
Processed animal proteins	15	0	0.0	8	0	0.0	14	3	21.4	17	0	0.0	30	0	0.0
Greaves	24	7	29.2				16	3	18.8	5	1	20.0	16	0	0.0
Egg shells - heat treated	53	0	0.0	67	0	0.0	15	0	0.0	6	0	0.0	12	2	16.7
Others	17	0	0.0	70	2	2.9	38	0	0.0	62	0	0.0	95	0	0.0
Total	**162**	**8**	**4.9**	**163**	**4**	**2.5**	**130**	**6**	**4.6**	**94**	**1**	**1.1**	**177**	**2**	**1.1**
Feed material of marine animal origin															
Fish meal	291	0	0.0	234	1	0.4	175	6	3.4	123	0	0.0	39	0	0.0
Fish oil	3	0	0.0				1	0	0.0	3	0	0.0		0	
Other fish products	50	0	0.0	22	0	0.0	16	0	0.0	12	0	0.0	4	0	0.0
Total	**344**	**0**	**0.0**	**256**	**1**	**0.4**	**192**	**6**	**3.1**	**138**	**0**	**0.0**	**43**	**0**	**0.0**
Feed material of cereal grain origin															
Barley and derived	175	0	0.0	178	0	0.0	101	0	0.0	305	1	0.3	105	0	0.0
Wheat and derived	1516	2	0.1	676	2	0.3	841	0	0.0	1052	1	0.1	471	1	0.2
Maize and derived	520	2	0.4	192	1	0.5	241	16	6.6	392	20	5.1	244	4	1.6
Others	141	1	0.7	152	0	0.0	50	0	0.0	337	0	0.0	125	0	0.0
Total	**2352**	**5**	**0.2**	**1198**	**3**	**0.3**	**1233**	**16**	**1.3**	**2086**	**22**	**1.1**	**945**	**5**	**0.5**
Feed material of oil seed origin															
Rape seed derived	4453	51	1.1	3971	19	0.5	2257	17	0.8	2276	20	0.9	334	7	2.1
Soya (bean) derived	5777	100	1.7	3081	41	1.3	2375	24	1.0	2531	18	0.7	1332	13	1.0
Sunflower seed derived	618	4	0.6	706	3	0.4	506	5	1.0	645	3	0.5	262	10	3.8
Linseed derived	53	0	0.0	46	1	2.2	9	0	0.0	22	0	0.0	4	0	0.0
Others	136	0	0.0	135	0	0.0	78	0	0.0	72	0	0.0	23	0	0.0
Total	**11037**	**155**	**1.4**	**7939**	**64**	**0.8**	**5225**	**46**	**0.9**	**5546**	**41**	**0.7**	**1955**	**30**	**1.5**

Category of feed materials	2008			2009			2010			2011			2012		
	# tested	# pos	SP (%)	# tested	# pos	SP (%)	# tested	# pos	SP (%)	# tested	# pos	SP (%)	# tested	# pos	SP (%)
Other feed material of plant origin															
Tubers and roots	171	1	0.6	100	0	0.0	31	1	3.2	23	0	0.0	9	0	0.0
Fruits and other seeds	129	1	0.8	139	0	0.0	67	0	0.0	26	0	0.0	70	0	0.0
Forages and roughages	27	1	3.7	21	0	0.0	19	0	0.0		0			0	
Rice and rice by-products	60	1	1.7	0			0			11	1	9.1	8	0	0.0
Vegetable fat and oil	12	0	0.0	0			0			6	0	0.0	4	0	0.0
Others	319	1	0.3	253	1	0.4	37	0	0.0	214	0	0.0	112	0	0.0
Total	**718**	**4**	**0.6**	**513**	**1**	**0.2**	**154**	1	**0.6**	**280**	**1**	**0.4**	**203**	**0**	**0.0**
Total for all feed materials	**15180**	**170**	**1.1**	**10616**	**71**	**0.7**	**7253**	**73**	**1.0**	**8744**	**66**	**0.8**	**3750**	**37**	**1.0**
Premixes	168	0	0.0	285	0	0.0	164	0	0.0	359	0	0.0	148	0	0.0

3.4 Salmonella serovars in compound feed and feed materials

In 568 Salmonella positive samples (127 samples of compound feed and 441 samples of feed materials) the Salmonella serovar has been determined. Multiple serovars can be present in one sample, however, multiple serovars were only determined in five of the 568 samples. In total 48 different Salmonella serovars were found. The most common serovars in compound feed were: Senftenberg, Mbandaka, Agona, and Livingstone, which accounted for 20.6%, 7.9%, 6.4% and 6.4%, respectively (out of the total of 127 cases in which at least one serovar was identified). Senftenberg was mostly found in compound feed of pigs and laying hens (10 each out of the 26 cases). Mbandaka was mostly found in compound feed of laying hens (6 out of the 10 cases). Furthermore, 6 out of the 8 cases of Agona, and 5 out of the 8 cases of Livingstone were found in compound feed of laying hens. Figure 16 shows the serovars found in compound feed during the five years.

In feed materials, the causative Salmonella serovar was not identified in 81 out of the 441 cases. When identified, the most common serovars were Senftenberg, Mbandaka, Livingstone, Cubana and Agona, which accounted for, respectively, 7.8%, 7.8%, 6.4%, 6.4% and 5.0%. Twenty one out of the 34 cases of Senftenberg were found in feed materials of oil seed origin, especially from rapeseed, soya (bean) and sunflower. Mbandaka was mostly found in feed materials of cereal grain origin (14 out of 34) and of oil seed origin (15 out of 34) of which byproducts of maize, rapeseed, soya (bean) were the main sources. Furthermore, 23 out of the 28 cases of Cubana were found in feed materials of oil seed origin. All the 22 Agona cases were found in feed materials of oil seed origin with rapeseed, soya (bean) and sunflower seeds and their byproducts as main sources. Figure 17 shows the serovars found in feed materials during the five years.

Out of the total of 568 samples, serovars related to foodborne human disease were: 30 (5.3%) Agona, 20 (3.5%) Enteritidis, 8 (1.4%) B. Java, 7 (1.2%) Infantis, 6 (1.1%) Hadar, 5 (0.9%) Typhimurium, and 1(0.2%) Parath. These serovars were mostly found in compound feed for laying hens and feed materials of oil seed origin. Even though the feed is contaminated with a serovar pathogenic to human, it does not imply the pathogen is transferred via the animal into the animal products resulting into human exposure.

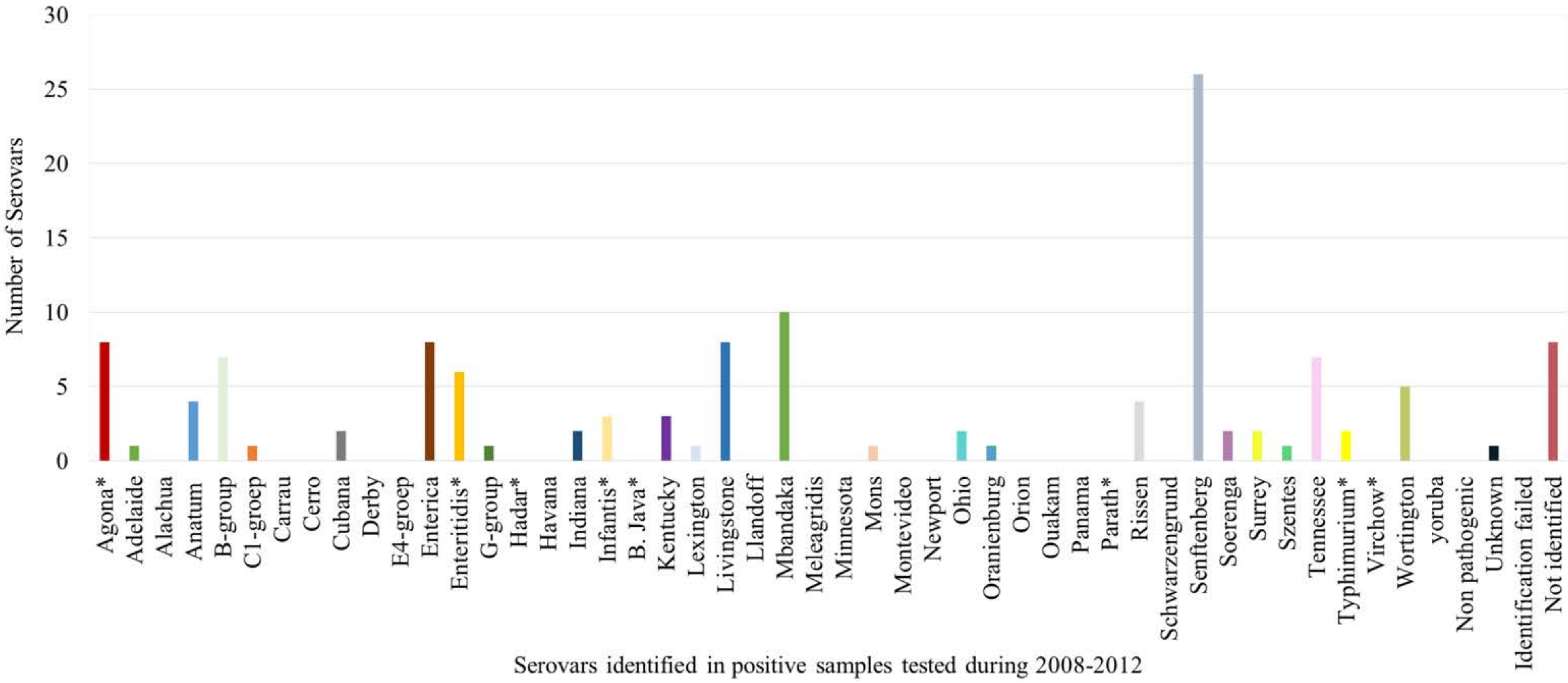

Figure 16 *Serovars of Salmonella identified in compound feed (positive for Salmonella) during 2008-2012.*

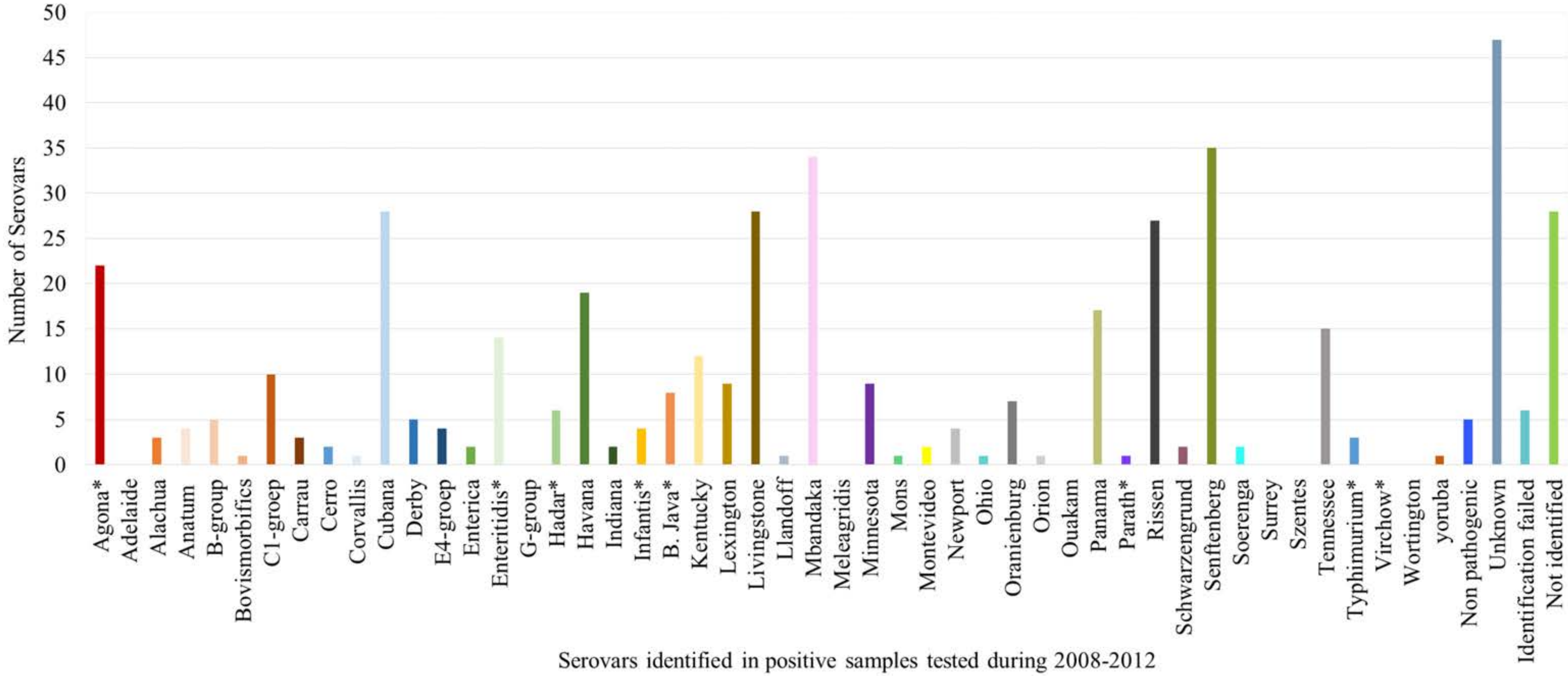

Figure 17 *Serovars of Salmonella identified in compound feed (positive for Salmonella) during 2008-2012.*

4 Discussion

Monitoring of *Salmonella* spp. in compound feeds, feed materials and pet foods is performed by most feed producers in many countries around the world with the aim to control the pathogen in the feed and food chain. *Salmonella* spp. can occur in all stages of the feed and food chain. Hence, the control of Salmonella in feed should be directed to all relevant sectors in the feed chain including the production, purchasing, processing and transportation of feed and feed ingredients all the way up to the farm level. Control at an early stage can prevent the spread of the pathogen along the chain. Accordingly, monitoring *Salmonella* spp. in feeds is important to minimize the prevalence of the micro-organism in the consecutive steps of the chain.

In this study, on average, an annual number of samples of about 10,000 compound feed and 9,100 feed materials were tested for *Salmonella* spp. during 2008 – 2012 and stored in the GMP+ monitoring database. This high total number of samples reflects the intensive monitoring program in the Netherlands. For nearly all categories of feed materials and compound feeds, however, the number of samples stored in the database decreased significantly during the five years. This decrease in number of collected samples might be related to the change in the production strategy in the Netherlands which is characterized by a decreasing number of feed companies (personal communication). Also, during the study years, results of samples in which Salmonella was not present were not stored in the GMP+ monitoring database (personal communication). In compound feeds, the highest number of samples tested for Salmonella was related to compound feed for poultry (54% of all compound feeds), in particular to compound feed for laying hens.

Based on the available (incomplete) data, considering all compound feeds together, no clear trend of S*almonella* spp. prevalence during the five years was seen. A decreasing trend of *Salmonella* prevalence was observed from 2008 to 2010, then the prevalence increased in 2011 reaching its highest level in 2012. The main contribution to the increased prevalence of *Salmonella* in 2012 was from compound feed for poultry, especially that for laying hens, as well as compound feed for pigs. Both compound feed for poultry and for pigs showed an increasing trend of Salmonella prevalence over the study years. However, not all ‘negative’ sample results were stored in the GMP+ monitoring database (personal communication). To simulate the missing negative sample data, the Salmonella prevalence was also calculated assuming that the total numbers of samples collected each year were the same as the number of samples collected in the first study year (2008) for these groups of compound feeds. In this scenario, the Salmonella prevalence decreased during 2009-2011, but increased again in 2012 to the same prevalence as in 2008 for compound feed for pigs, and to a higher prevalence as in 2008 for compound feed for laying hens (and for the entire group of compound feed for poultry). The increase over 2008-2012, which was significant in the current study, was not significant any more ($R^2 < 0.02$, data not shown) with the simulated results.

In feed materials, there was a large fluctuation in the Salmonella prevalence during the five years, with the highest Salmonella prevalence in 2012. In feed materials of land animal origin and of plant origin other than cereals and oil seeds, the Salmonella prevalence decreased significantly over the study years, whereas no significant trend was seen in Salmonella prevalence in feed materials of oil seed origin and of cereal grain origin. In general, maize and soy products, fish meal, meat and processed animal proteins showed a relative high prevalence of *Salmonella* spp. worldwide (Veldman *et al.*, 1995; Jones and Richardson, 2004). In accordance with those studies, current results showed that feed materials of land animal origin, marine animal origin (especially fish meal), cereal grain origin (especially maize and maize by-products), and oil seed origin (especially rapeseed, soya(bean) and sunflower) were the main sources of Salmonella prevalence during those five years.

In Europe, contaminated foodstuffs serving as a source for *Salmonella* spp. infection for human include turkeys (2.6 %), broilers (10.6 %), table eggs (17.0 %), and pigs (56.8 %) (EFSA, 2012). A significant decreasing trend in *salmonellosis* in human over the period 2008‑2012 has been reported by EFSA (EFSA, 2014). This reduction was assumed to be the result of the successful Salmonella control programs in poultry, particularly resulting in a lower occurrence of *Salmonella* spp. in table eggs (Graveland at al., 2013; Hugas and Beloeil, 2014; EFSA, 2014). The decreasing trend in *salmonellosis* in human coincided with the declining prevalence of *Salmonella* spp. in foodstuffs over the same period. In the Netherlands, a decrease of Salmonella prevalence was reported in broiler and pig meat in 2012 relative to 2008. The prevalence of Salmonella in fresh broiler meat at retail in 2012 was 6.6% as compared to 8.1% in 2008. The prevalence of Salmonella in fresh pig meat at retail in 2012 was 0% as compared to 4.1% in 2008 (Graveland *et al.*, 2013). Furthermore, during the past two decennia, the lowest Salmonella prevalence in table eggs was observed in 2009; this prevalence afterwards slightly increased during the years 2010 to 2012 (Graveland *et al.*, 2013).

In this study, a wide range of serovars has been reported from both compound and feed materials during the five years. Of all the serovars, *Salmonella* Senftenberg was the most prominent one in both feed sources. Only a few of the reported serovars were related to foodborne human disease. These include, in accordance to their presence, *Salmonella* Agona > Enteritidis > Infantis > Typhimurium in compound feed of all animals, and Agona > Enteritidis > Java > Hadar > Infantis > Paratyphi in feed materials. Even though a pathogenic serovar might incidentally occur in animal feed, this does not imply it poses a risk to human health.

Results of this study could be used by risk managers in the feed industry and at the government to evaluate the *Salmonella* monitoring program in feed and if necessary to make adjustments in relation to sampling of the most risky feed materials. This process is currently ongoing within the Netherlands.

5 Conclusion

Control of the presence of Salmonella in the feed chain is crucial in order to prevent the spread of *Salmonella* spp. infection in animals as well as human. Based on the available data, a high number of feed material samples is collected yearly for monitoring Salmonella presence in the Netherlands. The total number of samples tested for *Salmonella* spp. varied, however, between feeds and years, with a decreasing trend of total amounts of samples collected and stored in the GMP+ monitoring database during the study period (2008-2012).

In general, based on the dataset analysed in this study, *Salmonella* prevalence was highest in the last year (2012), especially in compound feed of pigs, and poultry (breeders and layers). From the feed materials, oil seeds, especially rapeseed, soya(bean) and sunflower, showed the highest prevalence of Salmonella in the study period, followed by greaves, fishmeal, and by-products of maize. However, except for maize (and derived) products, the absolute numbers of Salmonella positive sample results for these feed materials decreased over the study years, pointing towards a reduction of Salmonella presence, when similar amounts of samples were collected each year. The presence of Salmonella in milk and milk products, in compound feed of ducks, turkeys, horses, pets and other animals and in premixes was minimal to zero during those five years. In feed materials of land animal origin and of other plant origin, a significant decrease in Salmonella presence was seen over the years. Most of the Salmonella positive samples contained non-pathogenic serovars. Pathogenic serovars were only found in a limited number of samples, mainly from compound feed for laying hens and feed materials of oil seed origin. So, even when a feed material is Salmonella positive, the probability is it a pathogenic serovar is low.

Results of this study can be used by feed industry and the competent authorities for fine-tuning the monitoring program to those feeds in which the probability of the for the presence of *Salmonella* spp.,in particular pathogenic serovars, is highest. It is recommended to focus sampling on the above mentioned compound feeds and feed materials with the highest probability of being positive for *Salmonella* spp., in particular compound feeds for pigs and poultry, and the feed materials of oil seed origin, greaves and maize (derived). Sampling could be less intensive in the other groups of compound feeds and feed materials. It is also strongly recommended to store all monitoring results in the GMP+ database such to enable drawing more reliable conclusions.

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