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Detection and Isolation of *Salmonella* spp. in Animal Feeds from 2007-2011

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ABSTRACT

Salmonella species (spp.) are zoonotic pathogens that contaminate animal ingredients and finished feed and represent a significant human food safety hazard as identified by the Codex Animal Feed Taskforce. The United States (US) Food and Drug Administration (FDA) has promulgated regulations prohibiting *Salmonella* contamination in feed and has published a guidance document expressing their current strategy involving regulatory oversight of *Salmonella* contamination in feed. The Office of the Texas State Chemist (OTSC) initiated the broad surveillance of *Salmonella* spp. in 2007, in response to a *Salmonella enterica* serotype Typhimurium outbreak in frozen rodents, which are subject to the agency's regulatory oversight as defined by the Texas Commercial Feed Control Act. From calendar years of 2007-2011, 2622 total feed samples were collected and subsequently evaluated for *Salmonella* contamination using multiple screening methods, including polymerase chain reaction (PCR) and automated immunoanalysis. Three hundred and five out of 2622 samples were identified as being contaminated with *Salmonella* spp. representing 78 different serotypes. Since 2007, there has been a steady increase in *Salmonella* recovery rate, along with a corresponding increase in *Salmonella* serotype diversity. This increase in prevalence of *Salmonella*-contaminated feed stocks presents a potential risk to public health.

1.Introduction

Salmonella is a genus of gram-negative, rod-shaped, non-spore-forming *Enterobacteriaceae* with flagella. It is a highly diverse pathogen with more than 2,500 different serotypes. *Salmonella* spp. has been reported to be transmitted through contaminated food, water, and infected animals, resulting in in human illnesses across all age groups. Current comparative genomic research shows that *Salmonella* is characterized by high genomic plasticity (Carattoli *et al.*, 2005; Hochhut *et al.*, 1997). It has further been demonstrated that mobile elements play an important role in its evolution with larger plasmids conferring antibiotic resistance and virulence genes (Carattoli *et al.*, 2005; Hochhut *et al.*, 1997). In addition, *Salmonella* has been

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identified from bovine feces for 184-332 days at ambient conditions, as well as from avian feces for up to 28 months at ambient conditions (Inatsu *et al.*, 2004; Kearney *et al.*, 1993; Kim and Jiang, 2010; Lang *et al.*, 2004; Nicholson *et al.*, 2005; Oliveira *et al.*, 2011). These studies indicate that the main sources of soil-borne pathogens may be a combination of manure, water, and animal feces. Therefore, great caution and discretion may be required to manage risks associated with raw foods, including the application of various tools to verify process control, developing an intimate knowledge of microbial ecology within processing facilities, and focusing on improving process control for the detection of pathogens in end products.

From years 2006 to 2011, Centers for Disease Control and Prevention (CDC) have listed 21 *Salmonella* serotypes involved in 30 multiple state *Salmonella* outbreaks. The sample types vary from fresh produce, raw meat products, frozen entrée, manufactured food products, to small animals and animal food. These 21 different serotypes, including Agona, Altona, Baildon, Chester, Enteritidis, Hadar, Hartford, Heidelberg, I 4,[5],12:i:-, Johannesburg, Litchfield, Montevideo, Newport, Panama, Saint Paul, Schwarzengrund, Senftenberg, Tennessee, Typhi, Typhimurium, and Wandsworth (Behravesh *et al.*, 2010; Braden, 2006; Gupta *et al.*, 2007; Harris *et al.*, 2010; Medus *et al.*, 2006; Mody *et al.*, 2011; Smith *et al.*, 2008; Sotir *et al.*, 2009). Prior studies have examined the presence of *Salmonella* in animal feed and several have documented the relationship between the presence of *Salmonella* in feed to salmonellosis in animals and a possible link to human diseases (Barton, 2000; Crump *et al.*, 2002; Goldman, 2004; Hinton, 2000; Lee *et al.*, 2008; Loharikar *et al.*, 2012; Oosterom, 1991; Swanson *et al.*, 2007). The recent report of animal-originating *Salmonella* incidence resulting in a national *Salmonella* serovar Typhimurium outbreak, was from dogs that sickened over 575 humans nationwide in year 2009 in which the contaminated source was peanut putter also linked to human illness (CDC, 2009a; 2010). An FDA- Center for Veterinary Medicine (CVM) surveillance program of 2,058 complete animal feeds, feed ingredients, pet foods, pet treats, and supplements for pets during the same time period found the prevalence of *Salmonella* in these products to be around 12.5% (Li *et al.*, 2012).

The mission of the Office of the Texas State Chemist (OTSC) is to protect consumers and enhance agribusiness through a feed and fertilizer regulatory compliance program, surveillance and monitoring of animal-human health and environmental hazards, and preparedness planning. Since 2007, the OTSC has conducted an active *Salmonella* spp. surveillance program. This program was initiated in response to a *Salmonella* serovar Typhimurium outbreak among youth associated with handling of frozen rodents for feeding to pet snakes (Lee *et al.*, 2008). The OTSC's *Salmonella* testing and surveillance program represents a novel research with one of the most comprehensive *Salmonella* isolates collection from animal feed, as well as comparison of methodologies for screening *Salmonella* spp.

2. Materials and methods

2.1 Sample weighing and enrichment

OTSC collected and evaluated 2622 samples of feed ingredients and finished feed samples from calendar years 2007 to 2011. Animal feed samples were collected using sterile sampling techniques and delivered at next day by the courier service. Twenty-five gram of each feed sample (including waste-stream vegetable samples) was placed into a filtered stomacher bag. Two hundred and twenty-five milliliter (ml) of modified buffer peptone water (mBPW) enrichment media was then added to all samples and mixed by stomacher, or hand-mixing if necessary. Samples were swiftly moved to 37^{o}C incubator for 24 hours growth. Over this period, the screening methodologies used changed from the Neogen Reveal® test system to BAX® PCR-based detection (DuPont Qualicon Inc., Wilmington, DE) to the VIDAS immunoassay test system (bioMérieux Inc., Durham, NC).

2.2 Salmonella analysis- The Neogen Reveal® for Salmonella test system

Twenty-five gram of animal feed was mixed with Neogen REVIVE® medium and incubated at 37^{o}C for 4 hours. The Neogen Rappaport-Vassiliadis (RV) medium is added to each sample and incubated for 20~24 hours at 42^{o}C. One hundred and twenty microliters (µl) of sample culture enrichments were loaded into the sample port. The sample flows through the lateral flow testing device, providing distinct, visible results. The sample is negative for

Salmonella if the immunological developed signal appeared in the control zone, while presumptively positive if the signal appeared in the control and test zones (Bird *et al.*, 1999; Harrison *et al.*, 2006). All enrichments that showed a positive result with the lateral flow test device were processed through immunomagnetic separation (IMS) as described previously (Li *et al.*, 2010; Skjerve and Olsvik, 1991).

2.3 Salmonella Analysis- The BAX® PCR

The DuPont BAX® Q7 PCR instrument, utilizing the BAX® *Salmonella* PCR kit, was used to screen animal feed samples. Ten µl of each mBPW enriched culture was added into 500 µl of brain heart infusion (BHI) culture broth and incubated at 37°C for 3 hours. A lysate sample was prepared from each regrowth sample according to the BAX® *Salmonella* assay. The lysates were analyzed on the BAX® Q7 instrument with the BAX® *Salmonella* PCR kit (Cheung *et al.*, 2007; D'Aoust *et al.*, 2007; Koyuncu *et al.*, 2010; Silbernagel *et al.*, 2003; Tice *et al.*, 2009). All regrowth samples that had positive BAX® results were used to inoculate in the selective enrichment broth and then were processed through the IMS for further confirmation.

2.4 Salmonella Analysis- VIDAS assay

Ten ml selective broth (SX2) was mixed and inoculated with 100 µl of the overnight enriched mBPW culture media at 42°C for 24 hours. Overnight cultures were briefly mixed and 500 µl of each sample was pipetted into a bioMérieux VIDAS test strip. Each strip was placed onto the VIDAS Heat & Go block for 15 minutes and swiftly removed to cool for 10 minutes. One SLM strip and one solid phase receptacle (SPR) for each sample were loaded onto VIDAS for processing (Johnson *et al.*, 2009; McMahon *et al.*, 2004).

2.5 Confirmation of presumptive Salmonella samples

All OTSC samples that tested positive for *Salmonella* by Neogen Reveal®, BAX®, and VIDAS methods were also subjected to additional tests, which included traditional ChromID, Hektoen enteric (HE), Brilliant Green Selective media culturing, TSI/LIA slant identification, and API, to confirm results accuracy**.**

2.6 Traditional culturing

All screen positive SX2 cultures were briefly vortexed and streaked on the Chromogenic *Salmonella*, HE, and Brilliant Green/ Xylose lysine deoxycholate (XLD) selective agar plates. These inoculated selective media plates were then inoculated at 37°C for 24 hours. Typical *Salmonella* colonies showed pale pink to mauve in Chromogenic *Salmonella* media, while displaying pink to red colonies surrounded by pink to red medium in Brilliant Green Agar, and colonies are blue-green to blue colonies on HE plates.

2.7 Triple sugar iron agar (TSI)/ lysine iron agar (LIA) slant identification

Each isolate was inoculated by streaking and stabbing the TSI slant (one stab) and LIA slant (double stab). One additional tryptic soy agar (TSA) plate was streaked in tandem as one continuous operation. All slants and plates were incubated in a 37ºC incubator for 18-24 hrs. TSI and LIA slants were examined for growth and the reactions. TSI slants reactions show red in alkaline conditions and displays yellow in acidic conditions. LIA slants reactions show purple in alkaline conditions and displays yellow in acidic (negative) conditions (Knight *et al.*, 1990).

2.8 Salmonella biochemical confirmation- analytical profile index (API)

The API-20E test kit (bioMérieux Inc., Durham, NC) was used for biochemical identification of presumptive *Salmonella* spp. (Butler *et al.*, 1975; Murray, 1978). The screen positive isolates from TSA media plate were dispersed within an ampoule of 0.85% NaCl solution. These saline suspensions were swiftly transferred to testing capsules to incubate at 37ºC for 18-24 hrs. VP1, VP2, tryptophane deaminase (TDA), and James solution (5 gram 4-Dimethylaminobenzaldehyde, 25ml Hydrochloric acid, 75 ml 2-Methyl-2-butanol) reagents were added the next day as previously described (Akoachere *et al.*, 2009; Aldridge and Hodges, 1981; Swanson and Collins, 1980). Additional oxidase reaction was done separately by directly smearing the bacterial cultures onto the bactident oxidase test strips (EMD-Merck, Darmstadt, Germany). All color reactions were read from the tests and converted to a seven-digit analytical profile index code. The codes

from all tests were imported to the online API 20 E evaluation system. API 20 E - confirmed *Salmonella* isolates were further cultured and stored in TSB (tryptic soy broth) with 15% glycerol at -70°C.

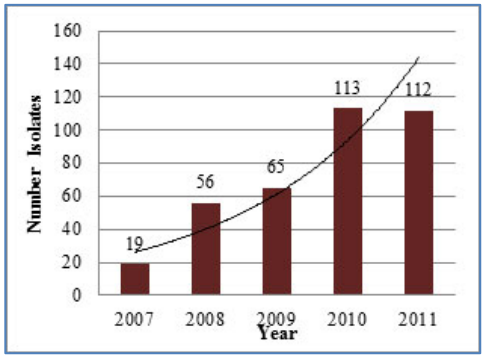
The *Salmonella* isolates were serotyped at the National Veterinary Services Laboratories (NVSL), Ames, Iowa. Pulsed field gel electrophoresis from NVSL were also analyzed by OTSC and entered into the CDC PulseNet database.

3. Results

3.1 Salmonella incidences in animal feeds

In 2007, eighteen out of 513 tested samples (3.5%) were reported positive and included 16 serotypes and 19 isolates (FIG. 1). In 2008, 2009, 2010 and 2011, 8.8%, 11.4%, 19.5% and 14.7% (Fig. 1 B) of the samples tested were *Salmonella* positive from 523, 507, 502, and 577 samples, respectively. The number of unique serotypes was 28 in 2008 through 2010 and 35 in 2011.Based on the number (FIG. 1A) and percentage (FIG. 1B) of the positive samples, we have observed the increasing *Salmonella* prevalence from our checked feed samples. The total numbers of *Salmonella* positive samples in 2011 (*n*=85) and 2010 (*n*=98) were much higher than that of 2009 (*n*=58) and 2008 (*n*=46) (FIG. 1A). The FIG. 1B also indicates an increasing ratio of *Salmonella* positive samples in recent years**.**

A.



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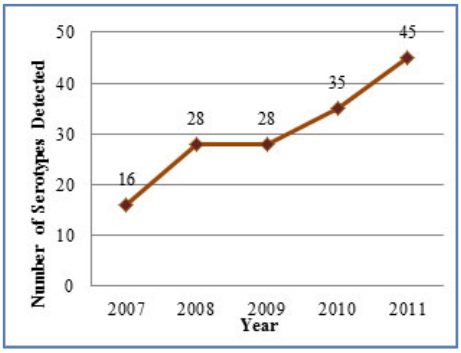


FIG. 1. *Salmonella* positive samples detected in animal feeds from 2007 to 2011. A) Total *Salmonella* positive samples detected from 2007- 2011 animal feeds. B) Percentage of the positive samples from the tested feed samples. The trend line from B) further indicated the increasing *Salmonella* contamination within the animal feeds between 2007 and 2011.

3.2 Serotyped Salmonella isolates from feeds

The number of unique *Salmonella* serotypes identified since 2007 (Fig 2) has consistently trended upwards. In 2007, there were 16 *Salmonella* serotypes identified from 19 *Salmonella* positive isolates (*n*=18, 3.5 % positive samples). Twenty eight different *Salmonella* serotypes identified from 56 positive isolates (*n*=46, 8.8 % positive samples) in 2008. In addition, there were 28 different *Salmonella* serotypes identified from 65 isolates (*n*=58, 11.4 % positive samples) in 2009 and 35 different *Salmonella* serotypes identified from 113 positive isolates (*n*=98, 19.5 % positive samples) in 2010. In 2011, 45 serotypes were detected from 112 positive isolates (*n*=85, 14.7 % positive samples) (FIG. 2). The data reveals diversity in *Salmonella* populations contaminating animal feeds. Among the serotypes identified, *Salmonella enterica* serovar Newport and serovar Dublin which are identified by the FDA guidance document as greatest concern in cattle feed, comprised 1.6% (*n*=6, *S.* Newport) and 0% (*n*=0, *S.* Dublin) of the isolates serotyped.

3.3 Salmonella prevalence in feed classes

The 2009-2011 feed samples were categorized under three specific product classes: animal protein products, 48.7% (*n*=74); beef cattle feeds, 16.7% (*n*=36); and cottonseed products, 26.7% (*n*=43) (FIG. 3B). Of all the *Salmonella* contaminated feed samples, 30.45% were from animal product proteins, representing a majority of samples testing positive. The ratio of animal product proteins to *Salmonella* positive samples was 22 out of 43 (51.16%) in 2011 and 39 out of 63 (61.90%) in 2010, which was 25-

35% higher than in 2009 (26.1%, 12 out of 46) (FIG. 3A).

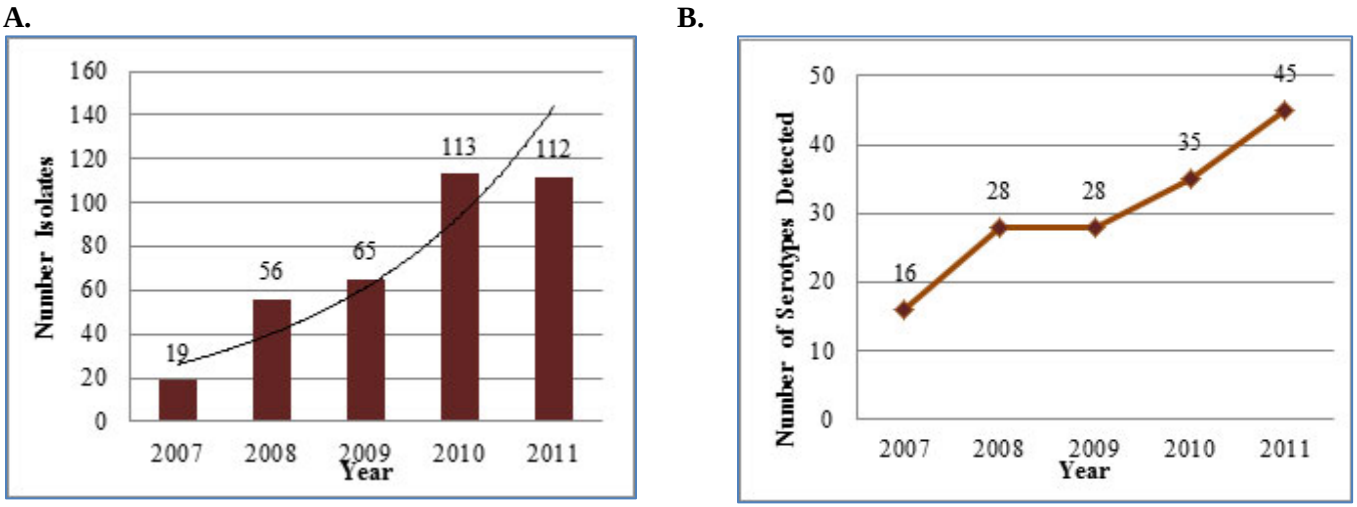


FIG. 2. Serological confirmation from the *Salmonella* positive isolates from 2007 to 2011. A) Number of *Salmonella* positive isolates with serological confirmation. The trendline reveals the exponential increase of the detected *Salmonella* isolates. B) Different *Salmonella* serotypes confirmed from the *Salmonella* positive isolates. All those serotypes have been shown in the Table 1.

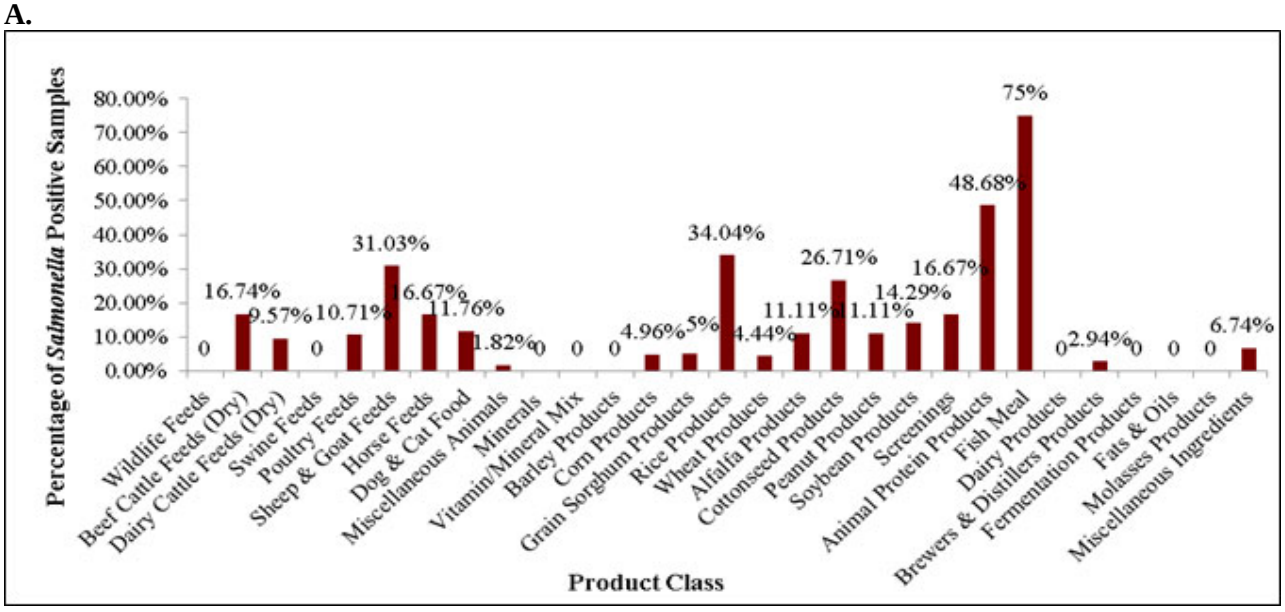
3.4 Novel methodologies and test accuracies

A few laboratories have compared the BAX® Q7 PCR assay and the VIDAS *Salmonella* (SLM) assay (Eriksson and Aspan, 2007). With the development of the reliable *Salmonella* detection methods in OTSC, basic simple statistics were presented to explain features of *Salmonella* recovery rate with respect to the calendar year, product class, and applied methodologies. Based on our *Salmonella* screening from 2010 and 2011, we further confirmed that there is no statistical difference between these two assays in detecting *Salmonella* spp. from most animal food matrices (data not shown). Both methods are reliable and accurate for *Salmonella* detection in animal feeds. By applying these methods, about double the amount of *Salmonella* false positive samples were eliminated compared to commercialized *Salmonella* testing kit (Fig 4). Low false-positive rates were found in the results from the BAX® PCR and VIDAS screening methods used in 2010 and 2011 (FIG. 4). From 2007 to 2009, the false-positive rates range from 65.7% to 80.2% of the Neogen Reveal® *Salmonella* test kit positive samples. In 2010, the BAX® PCR-based method only results in 1% false positive rate (99% positive). The VIDAS screening used in 2011 produced 4.5% false positive samples.

4. Discussion

Although samples varied among each calendar year (different annual plan of work), and first-line detection techniques changed, the trend lines of the data sets did provide some general information regarding the prevalence of *Salmonella* in animal feed. From 2007 to 2009, FDA food recalls have increased fourfold, arising most frequently from allergen, chemical, foreign material, and microbiological hazards and contamination (FDA, 2013). These sources have resulted in recalls in descending order, between 2007 and 2010. This is believed to be due to more sensitive detection techniques for *Salmonella*, *Escherichia. coli*, *Listeria monocytogenes*, and chemicals (Blossom *et al.*, 2009; Bowen *et al.*, 2007; Doyle *et al.*, 2009; Harris *et al.*, 2009; Perry *et al.*, 2007; Tate *et al.*, 2009).

A.



B.

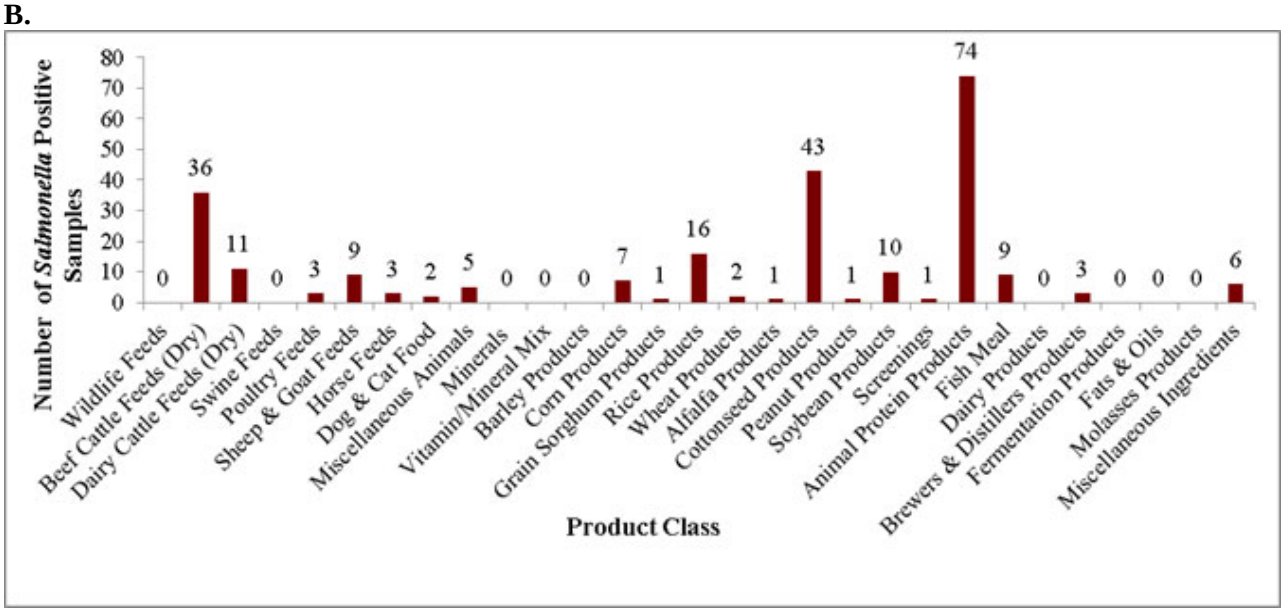


FIG. 3. Percentage of *Salmonella* positive samples by product class of the animal feeds (2009- 2011). A) The percentage of the *Salmonella* contaminated feed classes. B) Number of *Salmonella* positive sample detected from each feed class

There is limited information available on how the BAX® and VIDAS systems interact with matrices (Blackburn and McCarthy, 2000; Eriksson and Aspan, 2007). Recently, several laboratories indicated that the VIDAS system might be more reliable than the BAX® system based on the testing results and discussion through the Electronic Laboratory Exchange Network (eLEXNET) portal. The eLEXNET serves as a secure platform for multiple governmental agencies to participate in food safety activities, as well as compare findings and communicate. Therefore, OTSC initiated the VIDAS *Salmonella* screening study in 2011.

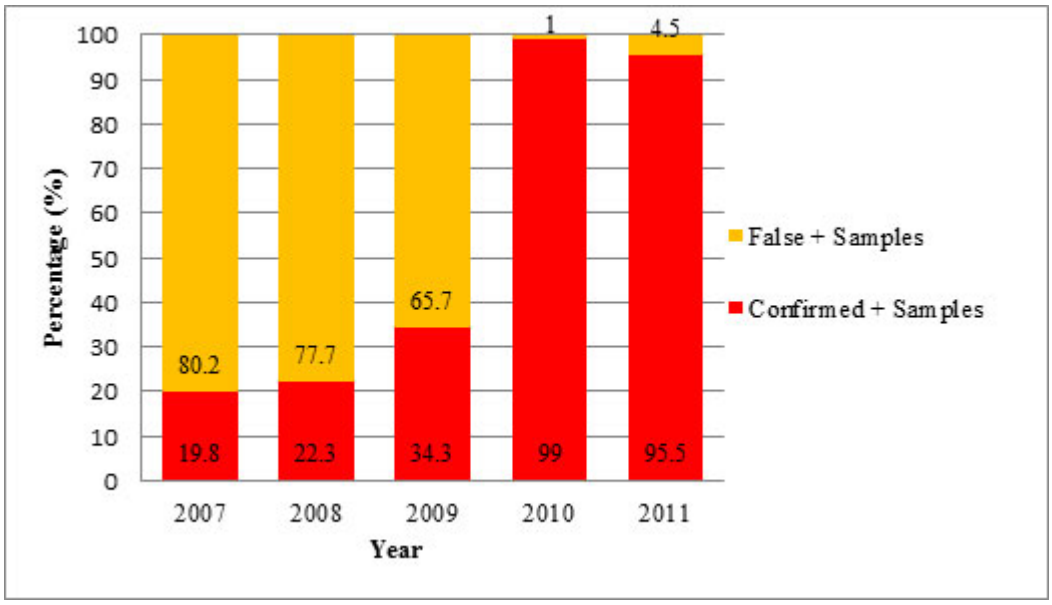


FIG. 4. Novel *Salmonella* detection method reduces the false positive samples. From 2007 to 2009, the *Salmonella* detection in feed was performed by Neogen Reveal® System resulting in false positive rates of 80.2%, 77.7%, and 65.7% in 2007, 2008, and 2009 respectively. In 2010, the BAX® PCR detection platform was used generating only a 1% false positive rate. In 2011, the VIDAS assay was utilized to screen for *Salmonella* and generated a 4.5% false positive result. Each sample comprising that 4.5% was further confirmed as a false positive by the BAX® PCR and serological studies. The accuracy of *Salmonella* detection has ascended since 2007 and statistically reliable results were achieved in 2010 and 2011. The numbers of the *Salmonella* serotypes were also analyzed from each calendar year. These false positives had been confirmed by BAX®, VIDAS, traditional culturing, and serotyping. According to this result, we confirmed that most of the failures of the BAX® and VIDAS assays, to identify *Salmonella* spp., appeared to be related to the matrix effect.

.Currently, the method best for *Salmonella* detection remains controversial. For the inspection of animal feeds both BAX® and VIDAS methods work well, without significant differences. All false positive results generated by the VIDAS screening were confirmed by BAX® PCR. False positive results generated by the BAX® PCR were confirmed by traditional culturing, IMS and enrichment, followed by an additional BAX® PCR screening. Based on these, we have confirmed that the VIDAS *Salmonella* and BAX® PCR assays are both good for *Salmonella* detection in animal feeds. In comparison to CDC multi-state *Salmonella* outbreak list and 2006-2010 OTSC *Salmonella* study in animal feed, nine out of 21 CDC-listed pathogenic serotypes were coincidentally detected in Texas animal feed (Table 1). Six out of these 9 serotypes from multi-state outbreaks were also detected in animal feed, as well as in the same year. Accordingly, great caution and discretion may be required to manage risks associated with raw foods, including the application of various tools to verify process control, developing an intimate knowledge of microbial ecology within processing facilities, and focusing on proving process control for the detection of pathogens in end products. Summarily, it is important to effectively and efficiently sample and test for *Salmonella* contamination to ensure food safety.

In addition, there were 28 new serotypes identified by OTSC since 2010 (Appendix 1) and 17 out of the 28 serotypes were identified in 2011, which are: Agona, Anatum, Cerro, Infantis, Johannesburg, Liverpool, Livingstone, Mbandaka, Meleagridis, Montevideo, Newport, Oranienburg, Orion, Orion var. O 15+, 34+ (Thomasville), Rissen, Schwarzengrund, and Senftenberg. Five out of these 17 serotypes, including Agona, Montevideo, Newport, Schwarzengrund, and Senftenberg, were initially reported in CDC multi-state outbreak from 2007 to 2011.

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Table 1. Timeline of *Salmonella* serotypes identified in CDC multi-state outbreak and OTSC feed.

Serotypes	Year of the CDC confirmed multi-state *Salmonella* outbreak	Year detected in OTSC feed samples
Agona	2008, 2011	2008-2011
I 4,[5],12:i:-	2007, 2010	2010
Johannesburg	2011	2009-2011
Litchfield	2008	2011
Montevideo	2009-2010	2007-2011
Newport	2009-2010	2009-2011
Schwarzengrund	2007	2008, 2010, 2011
Senftenberg	2009	2007-2011
Tennessee	2007	2008-2010

While no serotype constituted a majority of positive isolates, Mbandaka and Montevideo were the most frequently isolated from animal feed. As shown in the FIG. 5, Mbandaka is dominant in the 2009 to 2011 positive isolates (12%, 14%, 18% correspondingly; green slice) while Montevideo is 11% in 2010 and 9% in 2011 (orange portion). Additionally, 14% of confirmed serotypes were Senftenberg in 2009 (light blue marked) and 11% among the confirmed serotypes in 2010 were Infantis (purple slice).

Salmonella enterica serovar Mbandaka has a high detection rate as does Montevideo and Senftenberg. Montevideo and Senftenberg have been reported by CDC to cause human illness in different states. *Salmonella* serovar Mbandaka and other high incidence rate serotypes, such as Livingstone (19/365, 5.2%), Infantis (18/365, 4.9%), and Anatum (17/365, 4.7%) should also be carefully evaluated and monitored as to the level of threat they represent to public health.

It is unclear that these feed ingredients contributed to increased *Salmonella* outbreaks in animals and humans. Nevertheless, this hypothesis needs to be tested by monitoring the *Salmonella* population and serotypes in animals, animal feed, and even in the background environment. Based on this study, there were very diverse serotypes detected from animal feeds. The investigation of a *Salmonella* I 4,[5],12:i:- outbreak involving frozen rodents by Lee et al (2008) points out the importance of surveillance of *Salmonella* in animals parallel to investigating human illness. In fact, Montevideo and Senftenberg were two of the cases dominantly detected in feeds which were also reported in CDC *Salmonella* multi-state outbreaks.

By monitoring the *Salmonella* populations in animal feeds and the application of preventive controls including designation of a critical control point at the process step where control is most effectively applied, this biological hazard in feed can be reduced. The standard process will be established to minimize hazardous microbiological agents transmitted through the food chain and waste stream. Based on these results, OTSC will be able to strengthen national traceback systems, promote an outbreak response system that shortens the time between outbreak detection, resolution, and recovery, and improve methods for communicating with consumers about tracing foodborne illness outbreaks in the future.

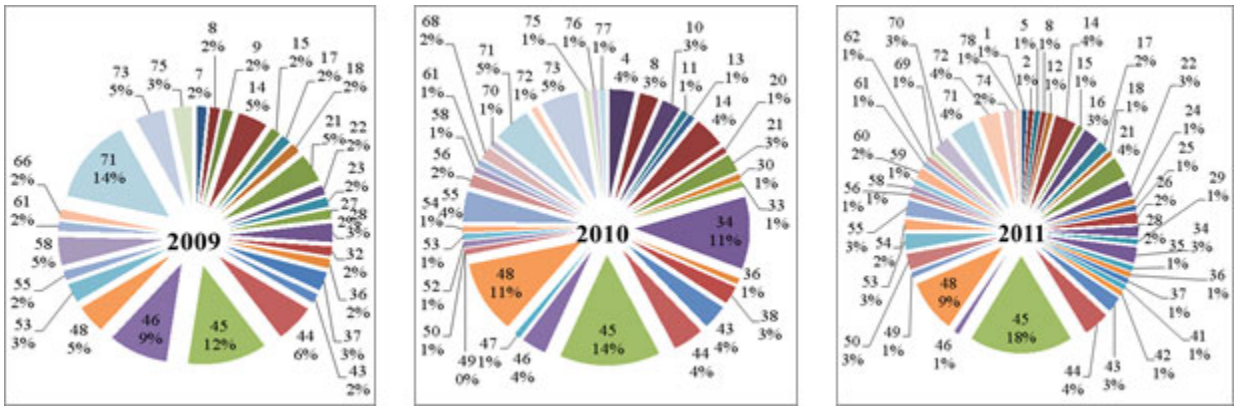

FIG. 5. *Salmonella* serotypes identified from *Salmonella* positive isolates (2009- 2011).
This figure reveals the source *Salmonella* serotypes for *Salmonella* incidences from 2009 to 2011. As also mentioned in the FIG. 2, there are increasing numbers of the serotype detected from 2009- 2011 (from 28 to 45 new serotypes identified). Those results also show very diverse distribution of the *Salmonella* serotype involved in each year. Notably, the serotype 45 (Mbandaka) is involved in more than 10% of incidences in all 3 years and serotype 48 (Montevideo) is involved in ~10 *Salmonella* incidences in 2010- 2011. In addition, serotype type 34 (Infantis) is involved in 11% incidences in 2010. In 2009, 14% of isolates have the serotype 71 (Senftenberg) and 9% for serotype 46 (Meleagridis). Serotype numbers and corresponding names are listed in Table 1.

5. Conclusion

This comprehensive screening through different animal feed classes in Texas was performed by OTSC and this article reports those results between 2007 and 2011. An increased recovery rate of *Salmonella* was found in animal feeds partially resulting from more adaptive isolation and detection techniques. Updates of the methodologies utilized to monitor the *Salmonella* in different feed matrices may impact our feed industry and ensure human and animal health. A dramatic reduction of false positive rates occurred as a result of using the BAX® PCR and VIDAS methods. In animal feed, a high number of *Salmonella* serovars Mbandaka, Livingstone, Infantis, and Anatum isolates were confirmed through multiple methods. Further real-time tracing or investigation should be performed to clarify the sources of contamination. Moreover, regimes of continual sampling and testing of animal feeds should be maintained, while phenotypic and genotypic characterization of confirmed *Salmonella* strains should continue.

Acknowledgements

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Appendix 1 . Timeline for identified *Salmonella* serotypes recovered from checked OTSC animal samples.

No.	Serotypes	07	08	09	10	11	Sub-Total	No.	Serotypes	07	08	09	10	11	Sub-Total
1.	21:-:e,n,x					1	1	40.	Lexington var. 15+, 34+ 9 (Illinois)	1					1
2.	3,19:-:z27		3			1	4	41.	Lille					1	1
3.	4,12: Nonmotile	1					1	42.	Litchfield					1	1
4.	42:z4,z23				4		4	43.	Liverpool		1	1	4	3	**9**
5.	6, 7:-:1, 5		1			1	2	44.	Livingstone	2	3	4	5	5	19
6.	6, 7:d:-		1				1	45.	Mbandaka	1	6	8	16	20	51
7.	8, 20 : poorly motile			1			1	46.	Meleagridis			6	4	1	**11**
8.	Agona		2	1	3	1	**7**	47.	Meleagridis var. O 15+ (Cambridge)				1		1
9.	Alachua			1			1	48.	Montevideo	1	7	3	12	10	33
10.	Amager				3		3	49.	Muenchen				0	1	1
11.	Amsterdam				1		1	50.	Muenster				1	3	4
12.	Amsterdam var. 15+		2			1	3	51.	Muenster var. O 15+ (Newhaw)		1				1
13.	Amsterdam var. O 15+, 34+ (Drypool)				1		1	52.	Muenster var. O 15+, 34+ (Arkansas)	1			1		2
14.	Anatum	2	3	3	5	4	17	53.	Newport			2	1	3	6
15.	Anatum var. O 15+ (Newington)			1		1	2	54.	Ohio				1	2	3
16.	Barranquilla					3	3	55.	Oranienburg		1	1	5	3	**10**
17.	Bergen			1		2	3	56.	Orion		1		2	1	4
18.	Braenderup		1	1		1	3	57.	Orion var. O 15+ (Binza)		1				1
19.	Brandenburg	1					1	58.	Orion var. O 15+, 34+ (Thomasville)	2	4	3	1	1	**11**
20.	Bredeney				1		1	59.	Ouakam	1	1			1	3
21.	Cerro	1		3	3	4	11	60.	Pomona					2	2
22.	Cubana		3	1		3	7	61.	Rissen			1	1	1	3
23.	Derby			1			1	62.	Roodepoort	1				1	2
24.	Ealing					1	1	63.	Rough O:b:e,n,x		2				2
25.	Gaminara					1	1	64.	Rough O:e:h,l,w		1				1
26.	Gera					2	2	65.	Rough O:y:1,5		1				1
27.	Give			1			1	66.	Rough O:z29:-			1			1
28.	Havana			2		2	4	67.	Rough O:z4,z23:-		1				1
29.	Hvittingfoss					1	1	68.	Rubislaw				2		2
30.	I 4,[5],12:i:-				1		1	69.	Ruiru					1	1
31.	I 6, 7:-:1,5	1					1	70.	Schwarzengrund		1		1	3	5
32.	I 6,7:k:-			1			1	71.	Senftenberg	1	2	9	6	5	23
33.	Idikan				1		1	72.	Soerenga				1	4	5

35.	Jodhpur					1	1	74.	Thompson					2	2
36.	Johannesburg			1	1	1	3	75.	Typhimurium var. O 5 - (Copenhagen)			2	1		3
37.	Kentucky			2		1	3	76.	Uganda				1		1
38.	Lexington	1	1		3		5	77.	Urbana				1		1
39.	Lexington var. 15+ (Manilla)		1				1	78.	Worthington					1	1
In total, *365 Salmonella* isolated with the 78 serotypes confirmed																