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Salmonella bacteria in farm animal feeds. Review

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SUMMARY

This review provides a data analysis of the test results for farm animal feeds. The analysis is based on the articles published from 1955 to 2020. It was found that the overall pooled prevalence estimates of *Salmonella* was 0.14: with a prevalence of 0.18 in raw feed components, 0.09 – in finished feed and 0.08 – in swabs from the surfaces of feed production equipment. The probability of contaminating raw animal feed components with *Salmonella* is 3.9 times higher than that for raw vegetable feeds. There is a tendency for *Salmonella* to be less detected in raw feed components; however, in finished feeds *Salmonella* detectability has remained unchanged for decades. The *Salmonella* prevalence in samples taken from environmental objects and surfaces of feed mill production equipment was 0.08. The risk of *Salmonella* detection at feed mills in the pre-heat treatment zone was 1.5 times higher than the risk of detection in the post-heat treatment zone. The analysis of *Salmonella* serovariants revealed that *S. senftenberg*, *S. montevideo*, *S. typhimurium*, *S. anatum*, *S. havana*, *S. enteritidis*, *S. cerro* are isolated everywhere. The *S. salford* serotype is found only on the African continent. A research into antimicrobial resistance of *Salmonella* isolates demonstrated resistance to such medicinal products as ceftriaxone, carbopenem and imipenem; and full genome sequencing showed at least one antibiotic resistance gene in 40% of *Salmonella* isolates detected at pig feed production plants.

Keywords: review, *Salmonella*, farm animal feed, *Salmonella* prevalence, antimicrobial resistance

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Бактерии рода сальмонелла в кормах для сельскохозяйственных животных. Обзор

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РЕЗЮМЕ

В обзоре представлен анализ данных по исследованию кормов для сельскохозяйственных животных, подготовленный на основании сведений из научных публикаций за период с 1955 по 2020 г. Установлено, что общая комбинированная оценка распространенности выявления сальмонелл составила 0,14 с распространенностью 0,18 в компонентах сырого корма, 0,09 – в готовом корме и 0,08 – в смывах с поверхностей комбикормового оборудования. Вероятность заражения сальмонеллой компонентов сырого корма животного происхождения в 3,9 раза выше, чем компонентов сырого корма растительного происхождения. Отмечена тенденция к сокращению выявления бактерий рода *Salmonella* в сырьевых компонентах кормов, в то время как выявляемость в готовых кормах остается неизменной на протяжении десятилетий. Превалентность сальмонелл при исследовании проб, отобранных с объектов окружающей среды и поверхностей оборудования комбикормовых заводов, составила 0,08. Риск выявления сальмонелл на предприятиях по производству кормов в зоне предтермической обработки был в 1,5 раза выше, чем риск обнаружения в зоне посттермической обработки. При анализе сероварианного состава сальмонелл установлено, что *S. senftenberg*, *S. montevideo*, *S. typhimurium*, *S. anatum*, *S. havana*, *S. enteritidis*, *S. cerro* выделяют

повсеместно. Серотип *S. salford* встречается только на территории Африканского континента. При изучении антибиотикорезистентности изолятов сальмонелл отмечена устойчивость к таким препаратам, как цефтриаксон, карбапенем и имипенем, а полногеномное секвенирование показало наличие по крайне мере одного гена антибиотикорезистентности у 40% изолятов сальмонелл, выделенных на заводах по производству комбикормов для свиней.

Ключевые слова: обзор, *Salmonella*, корма для сельскохозяйственных животных, распространенность сальмонелл, антибиотикорезистентность

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INTRODUCTION

Safety and quality of such food products as meat, milk and eggs depend on the safety and quality of animal feeds [1]. Livestock industry uses recipe-based mixed feeds produced at commercial feed mills. The feed industry is a complex structure consisting of feed mills, cereal-processing plants and production of protein and vitamin supplements [2]. Grain, grain-milling by-products, by-products of animal origin, vitamin and mineral supplements, as well as fats and oils are typical feed components. Pathogenic organisms, such as *Salmonella*, can enter a feed mill with raw feed components, survive processing and infect farm animals. They may further contaminate food for human consumption, thus, leading to outbreaks of foodborne toxicoinfections [1, 3–6].

Domestic or international movements of feed and feed ingredients for the trade purposes create risks for the spread of pathogens in new territories [7].

The World Organization for Animal Health has recognized that quality of animal feed has an impact on human and animal health and devoted a chapter in the Terrestrial Animal Health Code to this subject matter, named "The control of hazards of animal health and public health importance in animal feed" [7]. This section is completed by recommendations of the Codex Alimentarius Commission, set out in the "Guidance for Feed Risk Assessmen" [8].

The purpose of this work is to analyze data from scientific publications on *Salmonella* prevalence in mixed feeds and to characterize main *Salmonella* serotypes detected in raw feed components, on the surfaces of milling equipment and in finished feeds.

SAMPLING-ASSOCIATED RISK AND *SALMONELLA* DETECTION

Systematic errors made during studies of *Salmonella* prevalence are mainly associated with a sampling method. In practice, very few specialists make use of random sampling, more often samples are taken according to the willingness of subjects, i.e. feed mills, suppliers of raw feed components and retail outlets, to participate in the tests.

Parker E. M. et al. examined the sources of *Salmonella* detection and calculated the value of a systematic error associated with the sampling procedure [9]. Davies R. H. and Wray C. found that the dust collected from equipment surfaces and environmental objects in feed mills can be a *Salmonella*-representative sample of materials processed at the establishments [10].

Due to uneven distribution of *Salmonella* in a batch of raw feed components or finished feeds, several random subsamples shall be taken from the batch to make a representative pooled sample. Using the formula D. McChesney et al. [11]:

$$\frac{ln(a)}{ln(q)} = n,$$

where n – number of samples;
a – (1 – confidence interval);
q – (1 – expected prevalence),

calculated that 30 samples are required to detect *Salmonella* in the feed batch with a 95% probability, provided that 10% of the feed batch is infected with the pathogen:

$$\frac{ln(0.5)}{ln(0.9)} = 30.$$

Based on this formula for *Salmonella* detection, E. M. Parker et al. determined a detection probability (*DP*) for each study. The probability was calculated based on the number of samples collected to form a pooled sample for microbiological analysis [9]:

$$1 - e^{ln(q) \times n} = DP.$$

For example, if a pooled sample was prepared from four samples taken from a large amount of feed, the *DP* will be equal to:

$$1 - e^{ln(0.9) \times 4} = 0.34.$$

That is, the *Salmonella* detection probability will be 0.34, if it is present in 10% of the batch; whereas to ensure 95% confidence for bacteria detection, 60 samples must be taken, provided that only 5% of the batch are contaminated. In practice, only few researchers take so many samples. Thus, the reported *Salmonella*

prevalence in finished feeds often proves to be an underestimate of the true prevalence.

According to E. M. Parker et al., the *Salmonella* detection rate in all types of samples from 1955 to 2020 was 0.14 (0.11–0.17) with a confidence interval of 95%. It was noted that the sampling region had no impact on the *Salmonella* spread. However, over time, there has been a decrease in *Salmonella* prevalence in raw feed components [9].

Some authors explain a downward trend in *Salmonella* detection in raw feed components by introduction of good manufacturing practice (GMP) principles in feed mills, which include monitoring of microbial contamination of raw materials and finished product [12–14]. However, despite all the efforts to assess production conditions, the *Salmonella* prevalence rate (the ratio of the number of positive samples to the number of samples taken) in finished feeds has remained at the level of 0.09 for decades [9].

SALMONELLA IN RAW FEED COMPONENTS

The overall *Salmonella* prevalence in raw feed components was calculated by E. M. Parker et al. based on 67 tests and it was 0.18 (0.13–0.22) with a significance level of 0.95 [10]. Some authors compared *Salmonella* prevalence in components of animal and vegetable origin and found that the risk of *Salmonella* detection in raw feed components of animal origin was 3.9 times higher than the risk of *Salmonella* detection in raw feed components of vegetable origin (2.5–6.1 with a 95% confidence interval; $P < 0.001$) [15–23].

SALMONELLA IN FINISHED FEED

When analyzing the published data on *Salmonella* detection in finished feeds, the bacteria detection rate was calculated and it was 0.09 (0.06–0.11; a 95% confidence interval), that is, 2 times lower than in raw feed components. The researchers note that there was no difference in *Salmonella* detection in feeds for ruminants and feeds for monogastric animals (pigs, poultry) [3, 15, 16, 24–29].

SALMONELLA ON THE SURFACES OF FEED MILL PRODUCITON EQUIPMENT AND ENVIRONMENTAL OBJECTS

Environmental sampling at a feed mill and from the surfaces of equipment is a sensitive method of *Salmonella* detection [1, 30–33]. For environmental purposes and in order to protect equipment, mixed feed producers reduce the use of liquid disinfectants at the feed mills [34]. Therefore, thorough cleaning of equipment and production facilities is ensured by physical removal of organic substances (scraping) and dust. Dust on and around the equipment can be a convenient test object [10, 35]. Some researches demonstrate that *Salmonella* detectability on the equipment surface at the feed mills and in the environment is 0.08 (0.05–0.14 with a 95% confidence interval). It was found that the risk of *Salmonella* detection at feed mills in the pre-heat treatment zone was 1.5 times (1.03–2.17 with a 95% confidence interval) higher than the risk of detection in the post-heat treatment zone for the raw mixed feed materials [3, 16, 26, 36–39].

Infected raw feed materials are the main source of environment and equipment contamination. Therefore, based on a risk-oriented approach it is appropriate to assume that all raw feed components are infected, thus, corresponding microbiological safety control shall be ensured [35].

Good manufacturing practice at a feed mill is based on the division into zones in which raw feed components are stored and processed (dirty zone) and feed packing zones (clean zone) [28]. Such division enables to control movements of personnel, equipment and air from dirty to clean areas of the establishment. In addition, *Salmonella* that has got into high-fat feed with low water activity, can survive the heat treatment, enter the growth phase in warm and humid conditions and form biofilms on the surfaces of equipment, which can potentially become a source of feed contamination [40–42].

SALMONELLA SEROVARIANTS

From 1955 to 2020, the scientific literature provides data on 19,690 *Salmonella* isolates recovered from feed and feed components, 334 different serotypes were identified among them. The main detected serotypes are summarized in the Table. The data suggest that most serotyped isolates were studied in the European countries (59%). The isolates recovered from feeds in the American continent account for 17%, Africa – 12%, the Western Pacific region – 10%, the Eastern Mediterranean – 0.8%, Southeast Asia – 0.7%. However, despite significant imbalances in the number of detected *Salmonella* serotypes in laboratories of various countries, such serovariants as *S. senftenberg* (11% of the total number of serotyped isolates), *S. montevideo* (3.8%), *S. typhimurium* (3.7%), *S. anatum* (3.4%), *S. havana* (1.6%), *S. enteritidis* (1.3%), *S. cerro* (0.97%), are found on all continents. At the same time, *S. salford* serotype was recorded only on the African continent.

S. typhimurium bacteria are widespread all over the world and are most often found in pigs and pork products, whereas *S. enteritidis* is detected in poultry, and *S. anatum* is detected in beef. Such serotypes as *S. agona*, *S. montevideo* and *S. infantis* are also among the ten most frequently detected in animal feeds, and are also associated with foodborne diseases in humans [43, 44]. *S. enteritidis* is the most frequently detected *Salmonella* serotype, which is of public health importance in the USA and Europe, and it is the second important in Australia [45]. Thus, the *Salmonella* serovariant landscape established for feeds corresponds to the diversity of the main *Salmonella* serotypes detected in tests of livestock products and during investigation of salmonellosis cases in humans.

ANTIMICROBIAL RESISTANCE OF *SALMONELLA*

The scientific literature provides information on testing antimicrobial sensitivity of 1,735 *Salmonella* isolates recovered from feeds [27, 46–51]. It is reported that among the isolates recovered on the African continent, 75% were resistant to ceftriaxone, a third-generation cephalosporin commonly used to treat severe cases of salmonellosis in humans [52, 53].

It is believed that the use of cephalosporin in livestock production and healthcare creates good competitive surroundings for microorganisms resistant to carbapenems [54]. There have been reports on isolates resistant to carbapenem and imipenem, which are recognized as the "last-resort" treatment for some bacterial infections, and

Table
***Salmonella* serotypes isolated from feed, feed components, equipment and environmental objects of feed mills for the period from 1955 to 2020** (according to E. M. Parker et al. [9])

No.	Serotype	Number of identified isolates by region						
		Total	Europe	America	Western Pacific (China, Japan, Korea, New Zealand, Australia Mongolia, Vietnam)	Eastern Mediterranean (Afghanistan, Egypt, Iran, Iraq, Morocco, Syria, Pakistan, Tunisia)	Southeast Asia (Bangladesh, India, Nepal, Thailand, Indonesia)	Africa
1	*S. mbandaka*	2,348	2,035	126	111	0	2	74
2	*S. senftenberg*	2,187	1,671	285	184	4	7	36
3	*S. tennessee*	2,072	1,748	133	183	5	3	0
4	*S. agona*	1,374	1,179	56	136	0	3	0
5	*S. montevideo*	758	376	327	10	6	2	37
6	*S. typhimurium*	734	551	45	61	5	33	39
7	*S. anatum*	676	268	146	206	3	9	39
8	*S. infantis*	522	298	87	61	0	1	71
9	*S. binza*	506	250	127	3	2	0	0
10	*S. salford*	458	0	0	0	0	0	458
11	*S. schwarzengrund*	360	65	55	8	0	3	229
12	*S. cubana*	325	147	134	43	1	0	0
13	*S. havana*	323	157	21	84	2	4	55
14	*S. livingstone*	298	184	62	43	9	0	0
15	*S. enteritidis*	255	197	16	31	1	4	6
16	*S. orion*	228	21	49	77	0	3	78
17	*S. cerro*	191	14	121	40	5	1	10
18	*S. oranienburg*	190	44	114	20	0	0	12
19	*S. eimsbuettel*	188	13	134	41	0	0	0
20	*S. bredeney*	183	46	123	13	1	0	0
Total serotyped isolates		19,690	11,664	3,379	2,011	160	140	2,331

resistance to carbapenems is considered a serious public health problem [55]. Full-genome sequencing of *Salmonella* isolates found at feed mills producing pig feeds has shown that 40% of them have at least one antibiotic resistance gene [56]. These results indicate that antibiotic resistant microorganisms may spread among animals through feed and its components.

CONCLUSION

Contamination with *Salmonella* bacteria during feed production remains a significant problem of veterinary medicine and public health.

The overall *Salmonella* prevalence in mixed feed production has decreased since 1955, which is associated with a drop in bacteria detections in raw feed components. The *Salmonella* detectability in finished feeds, on the surfaces of milling equipment and on environmental objects has not changed over 50 years of observations. Microbiological control in feed production should include sampling from the environment in addition to sampling of raw feed components and finished feeds. Dust and mixed feed samples taken from the surfaces of the milling equipment and around it are a good object for tests, ensuring sensitivity of the *Salmonella* detection method. The number of samples taken from feeds and raw feed components shall be calculated based on the desired level of confidence and the estimated level of contamination. *Salmonella* serotypes detected in samples from feed mills equipment, from mixed feeds and raw feed components correspond to the serotypes detected in farm animals and in salmonellosis cases in humans.

Among the *Salmonella* isolates recovered from feed samples, there are bacteria resistant to modern antibiotics, which are "critically important" for medical use according to the classification of the World Health Organization.

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