OPEN ACCESS

GAVIN PUBLISHERS

Archives on Veterinary Science and Technology

Holzhauer M, et al. Arch Vet Sci Tecnol: AVST-143.

Doi:10.29011/AVST-143/100043

Case Report

A Remarkable Clinical Outbreak of *Salmonella enterica* subsp. *enterica* serovar Typhimurium variant 1,4,[5],12:i:- in Dairy Cattle

Menno Holzhauer[1*], Reinald Duijn[2], E Olde Heuvel[3], Annet Heuvelink[1]

[1]GD Animal Health, Deventer, The Netherlands

[2]Vallei and Wetering Practice, Renswoude, The Netherlands

[3]The Netherlands Food and Consumer Product Safety Authority, The Hague, The Netherlands

[*]**Corresponding author:** Menno Holzhauer, GD Animal Health, Deventer P.O. Box 9, 7400 AA Deventer, The Netherlands. Tel: +31570660364; Email: m.holzhauer@gdanimalhealth.com

Citation: Holzhauer M, Duijn R, Olde Heuvel E, Heuvelink A (2018) A Remarkable Clinical Outbreak of *Salmonella Enterica* subsp. *Enterica* serovar Typhimurium variant 1,4,[5],12:i:- in Dairy Cattle. Arch Vet Sci Tecnol: AVST-143. Doi:10.29011/AVST-143/100043

Received Date: 28 March, 2018; **Accepted Date:**10 April, 2018; **Published Date:** 25 April, 2018

Abstract

This case report describes a dramatic presentation of a *Salmonella* outbreak in a herd of 110 dairy cows. Almost at the same moment serious clinical problems were diagnosed in 90% of the investigated cows. All cows were slow, inactive, showed serious decrease of feed intake, diarrhoea with fresh blood clots and high fever (> 40.5 °C). Finally 17 cows were so weak that they died or had to be euthanized within a period of 3 weeks after the start of the clinical problems. After culturing the faecal samples were typed as *Salmonella enterica* subsp. *Enterica* serovar Typhimurium variant 1,4,[5],12:i:-and milk samples serological typed as *Salmonella* group B. Above that, some employees had very serious enteritis problems, whereby the same monophasic *S. typhimurium* strain as the causative agent was found. Based on exclusion of other sources, the infection was most possibly introduced by contaminated concentrate. This report describes the clinical and laboratorial investigation and discusses how to perform the sampling of the suspected feed by a responsible officer in such cases. Above that, this report confirms the necessary very strict hygienic measures to limit transmission to staff and family members.

Keywords: Dairy Cattle; Massive Outbreak; *Salmonella typhimurium*; The Netherlands; Zoonosis

Introduction

Salmonella enterica subsp. *enterica* serovar Typhimurium monophasic 1,4,[5],12:i:- (*S. typhimurium)*is of increasing importance as a cause of human salmonellosis. In 2014, 7.8% of all reported confirmed cases of human *Salmonella* is in the European Union/European Environment Agency were caused by this serovar [1]. In the Netherlands, the proportion of confirmed human salmonellosis cases caused by monophasic *S. typhimurium* 1,4,[5],12:i:- increased from 2% in 2005 to 21% in 2014. In 2014, approximately 20% of human salmonellosis cases in the Netherlands were attributed to cattle as the source of infection [2].

The incidence and prevalence of monophasic *S. typhimurium* 1,4,[5],12:i:- in cattle are unknown. The proportion of *Salmonella* isolates from post mortem samples from Dutch dairy herds that were typed as serogroup B (to which monophasic *S. typhimurium* 1,4,[5],12:i:- belongs) increased from 11% in 2005 to 47% in 2014 (unpublished observations, GD Animal Health). To reduce any potential risk of cattle as a source of human monophasic *S. typhimurium* 1,4,[5],12:i:- infections, it is important that outbreaks in cattle are recognised early. However, the clinical picture of bovine *S. typhimurium* 1,4,[5],12:i:- infections is not well defined. Therefore, in this paper, an unusual presentation of a severe clinical outbreak in a dairy herd and some employees caused by a monophasic *S. typhimurium*1,4,[5],12:i:- strain is described, inclusive an advise how to secure suspected materials.

Herd

The dairy herd, visited October 1st 2015, was a private family herd with 2 young kids and 2 part time employees, was located in the province of Utrecht, in the centre of the Netherlands and consisted of 110 dairy cows, a 2.5 years old breeding bull and 101 young stocks. The herdsman applied not any preventive treatment, no cattle was purchased and the cattle was permanent housed, so there was not any contact with neighbouring herds. The herd had no assurances for catastrophes. Cows were milked in two Auto-

matic Milking Stations (AMS). Dry cows and young stock (> 8 months) were housed in the same barn and fed the same roughage as the milking cows. Based on kilogram dry material, the ration of the milking cows consisted of grass silage 6, corn silage 3, soybean meal 1, brewer's grains 1, sugar beet pulp 2 and concentrates 2 to 9 per head per day. The silages and the beet pulp were stored outside, covered by plastics and the monitor the presence of wild animal's heat cameras was present. Concentrates, mainly based on grain products, were fed in the AMS. The remainder of the ration was as mixed ration fed in front of the feed rake. Young stock above 1 year of age and dry cows were fed roughage (grass and corn silage mixed with the feed remnants of the dairy cows) without concentrates. The concentrate plastics silo (content 30 kg tons) had been refilled three days before the onset of clinical signs, and at that moment of filling about a quarter of the concentrate was left in the silo. Prior to the disease outbreak, the health status of the herd was Leptospirosis certified free, BVDV certified free, Johne's disease and Salmonellosis unsuspected. The herd had no previous experience with Salmonellosis and applied no vaccination on cattle and farm personal experienced not any diarrhoea problems in recent years. Normally no visitors but the veterinarian and feeding advisors visited the herd, always wearing herd coveralls and boots.

Disease Outbreak

Clinical signs were first recognized when four dairy cows showed as ever decrease of > 50% feed intake (concentrate, grass and corn silage), milk production drop, diarrhoea and fever (40-41°C). One day later, based on information of the AMS approximately 80% of the cows showed a decreased feed intake, a drop-in milk production and/or fever (> 40°C, measured in the AMS). The next day, overall roughage intake of the milking cows was further reduced to approximately 20% of the original intake, diarrhoea without blood or pseudo-membranes was seen and some cows showed watery nasal discharge. Three days after the onset of clinical signs, the first author visited the herd and was confronted with a dramatic presentation.

Results

Almost all cows were seriously ill, had body temperatures of 40.5 - 41.2°C, watery diarrhoea with fresh blood and pseudo-membranes (Figure 1) and a >50% reduction of feed intake at the feeding rake. Cows were inactive and hardly ruminating; the average milk production had dropped from 28 to 9 litres. Two cows were euthanized because of the severity of their clinical signs. Remarkably, no clinical signs were observed in any of the dry cows or young stock. During the following days, there were no signs of transmission of the infection to the young stock on the farm until 2 weeks after the start of symptoms in dairy cows. On day 4 of the outbreak, the concentrate was temporarily stored in new big bags, and no longer fed. The silo was filled again with a new batch of concentrates. Clinical signs (diarrhoea, fever and reduced feed intake) were seen for about 10-14 days after removal of the concentrate. In most cows, milk production did not (fully) recover, especially cows above 100-120 days in milk. In total, during the disease outbreak of 14 days, ten cows died or had to be euthanized due to complete physical collapse and seven other cows aborted (Table 1). In May 2016 the same amount of milk was delivered to the milk industry. Three farm employees had fever (40, 5°C), diarrhoea, dehydration around day 7-10 after the onset of the clinical signs in the milking herd.

Figure 1: Manure of sick cows with fresh blood and pseudo membranes present during an outbreak of *Salmonella typhimurium.*

25-9-2015	27-9-2015	30-09-2015	2-10-2015	06-10-2015	8-10-2015	21-10-2017
new concentrate	onset of clinical symptoms: milk drop, stop eating, general illness, nasal discharge, diarrhoea	visiting herd, 90% of cows fever, sick, severe diarrhoea with pseudo-membranes and blood included sampling blood and manure for serology and bacteriological investigation	removal of concentrate	*Salmonella enteritica* subsp *enterica* serovar Typhimurium proven in the lab. BVDV not proven	gradual repair of production of fresh cows mainly, start clinical problems in calves	end of clinical symptoms
loss of 10% of dairy cows related to bacterial septicaemia, 7% abortion not any clinical problems in young stock, until 2 weeks after start in dairy, although received almost same roughage, but not same concentrate						

Table 1: Overview of the progress of a typical and very serious *Salmonella enteritica* subsp. Typhimurium outbreak in a Dutch dairy herd.

Collection of Samples

During the first herd visit, four faecal samples, blood samples and nasal swabs were taken from 5 affected cows. Three weeks later, 11 individual milk samples were taken for *Salmonella* antibody detection. Samples from suspected concentrate stored in the big bags and from several production stages at the concentrate producing factory were collected by the Dutch Food and Consumer Product Safety Authority. Stool samples of 1affected farm employees were sent by their GP to a regional laboratory for bacteriological examination. Seven months after the outbreak (May 2016) the farmers tested 5 heifers (young stock in their 2nd year of life during the outbreak) and 5 calves, both serologically and all herds in The Netherlands are standard monitored for the presence of *Salmonella* antibodies (B/D Elisa) by the milking industry every 3 months.

Laboratorial Investigation

Nasal swabs were tested using a bovine herpes virus 1 -PCR at the GD laboratory (Inhouse real time PCR) [3]; viral DNA could not be detected. Faecal samples were used for direct culture on **x**ylose lysine deoxycholate (XLD) agar and from all four samples *Salmonella* sp. was isolated with the antigenic structure 1,4,[5],12:i:-. After culturing and typing *Salmonella* group B by the GD, the antigenic typing of the isolates, was confirmed by the RIVM as monophasic *S. typhimurium*, with a luminex technology multiplex assay, showing 35 H and 7 O antigen [4]. The isolates were characterized by MLVA profile 03-14-09-00-211.Bacteriological examination of the concentrate and subsequent typing of the isolates revealed the same *Salmonella* serotype and genotype as the isolates from the faecal samples. In all 11 milk samples *Salmonella* antibodies were detected using an in-house *Salmonella* Serogroup B/D antibody ELISA. In the stool of affected employee *Salmonella* spp. was cultured by the RIVM.

Examination of the blood samples in May 2016 were all not proven, which meant no transmission within the herd to young stock or calves. Monitoring *Salmonella* serology of the bulk milk showed the presence of antibodies until August 2017.

Discussion

In our experience, this was a remarkable, massive *S. typhimurium* infection (monophasic variant); in dairy herds the infection generally spreads less extensively among susceptible populations [5] and secondly because *Salmonella dublin* (*S. dublin*) outbreaks are normally more prevalent and clinical signs are more severe than *S. typhimurium* outbreaks [6]. The presence of serious clinical symptoms of *Salmonella* enteritis among three of the employees was in line with other *Salmonella* outbreaks in dairy herds [7]. The course of the outbreak in the herd suggests a point source exposure, with the focus on the concentrate, as the presumptive source of the infection, and delivered three days before the onset of the symptoms. Although the same *Salmonella* type was found in this concentrate, a causal relationship could not be proven, since the samples collected on the farm were taken from open big bags, where secondarily contamination never can be excluded. Samples collected during the concentrate production process at the feed mill were negative for *Salmonella* spp. (The Netherlands Food and Consumer Product Safety Authority, personal communication). The repair of the production, based on liters delivered to the milk industry took almost a year, but one should realize that the (loss of) production was a consequence of less milk by sick cows, a loss of 10 cows, loss of production due to abortion and introduction

of new heifers. Although transmission of *Salmonella* within herds is most times limited and in most cases related to *S. dublin*, such massive outbreaks caused by *Salmonella* contaminated feed have been described before. In England with *Salmonella* Newport, as the causing agent, most cattle seemed to rid themselves of infection during the following months whether they were at pasture, housed in cubicles or in byres. Some cows excreted *Salmonellas* for up to 11 months after the disease outbreak. Associated human salmonellosis was confirmed on 3/41 (7%) of the farms [8]. This case report describes a very serious outbreak of *Salmonella* in a dairy herd inclusive some enteritis problems of employees, with a monophasic *S. typhimurium* strain as causative agent, possibly introduced by contaminated concentrate. In case of outbreaks where feed is suggested as a point source of the infection, timely and thorough sampling of the suspected feed by a responsible officer is therefore of great importance to prove the causal relationship. Above that, very strict hygienic measures are advisable to limit transmission to staff and family members.

Conflict of Interest Statement

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

References

1. Anon (2015) The European Union summary report on trends and sources of zoonoses. zoonotic agents and food-borne outbreaks in 2014. EFSA Journal 13: 4329.

2. Zomer T, Kramer T, Sikkema R, De Rosa M, Valkenburgh S, et al. (2015) Staat van zoonosis. Rivm Repository: 2014.

3. Wernike K, Hoffmann B, Kalthoff D, König P, Beer M (2011) Development and validation of a triplex real-time PCR assay for the rapid detection and differentiation of wild-type and glycoprotein E-deleted vaccine strains of Bovine herpesvirus type 1. Journal Virological Methods174: 77-84.

4. Wuyts V, Roosens NH, Bertrand S, Marchal K, De Keersmaecker SC (2015) Guidelines for optimization of a multiplex oligonucleotide ligation-PCR for characterization of microbial pathogens in a microsphere suspension array. Biomed Research International: 790170.

5. Visser IJ (1994) An outbreak of *Salmonella dublin*. Tijdschrift voor Diergeneeskunde 119: 101.

6. Van Schaik G, Klinkenberg D, Veling J, Stegeman A (2007) Transmission of *Salmonella* in dairy herds quantified in the endemic situation. Veterinary Research 38: 861-869.

7. Kagambèga A, Lienemann T, Aulu L, Traoré AS, Barro N, et al. (2013) Prevalence and characterization of *Salmonella enterica* from the feces of cattle, poultry, swine and hedgehogs in Burkina Faso and their comparison to humans isolates. BMC Microbiology 13: 253.

8. Richardson A (1975) Outbreaks of bovine salmonellosis caused by serotypes other than *S. dublin* and *S. typhimurium*. The Journal of Hygiene (London) 74: 195-203.