Salmonella enterica serovar Dublin infection in dairy cattle: a case study on the management of an outbreak in Italy

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SUMMARY

Salmonella enterica subsp. enterica serovar Dublin (S. Dublin), is a serovar adapted to cattle, causing both intestinal and systemic infections. The introduction of the bacterium leads to serious economic losses due to abortions, high mortality in calves and persistent infections, also representing a major health problem as zoonotic agent. The aim of this study was to describe an outbreak of S. Dublin on an Italian dairy cattle farm and to assess the effectiveness of the management protocol prepared by the Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe).

S. Dublin was diagnosed on a cattle farm in Northeastern Italy following the conferral at the IZSVe of a newborn calf that died from enteric syndrome. At the autoptic exam pathological findings were observed in gut, liver, pericardium, lungs, joints, lymph nodes and abomasum. Considering the pathogenesis of S. Dublin, authors decided to apply a protocol prepared by the IZSVe based both on direct and indirect prophylaxis. Particularly, an autogenous vaccine against S. Dublin prepared by the Istituto Zooprofilattico Sperimentale della Sardegna (IZS Sardegna) was administered.

Screening tests were performed on fecal and milk samples (bulk tank milk) and on environmental swabs from lactating and dry cows' boxes.

A pre and post-vaccination screening in 3-times (T0, T1, T2) was performed on serum, feces and milk to assess the immunization of cows and the effectiveness of the protocol itself. The first sampling took place 1-day prior immunization, the second and the third 2 and 11 months later respectively.

Serological examination identified 25%, 100% and 73% positive animals at T0, T1 and T2 respectively. No fecal sample in all time-points was found positive. After vaccination only 1 milk sample turned out positive.

Considering the pathogenesis of S. Dublin, the negativity of the bacteriological exams suggests a positive effect of the protocol in the reduction of clinical cases, circulation of the etiological agent and biocontainment of the infection.

KEY WORDS

Salmonella; Dublin; Cattle; Autogenous vaccine.

INTRODUCTION

Salmonella enterica subsp. *enterica* serovar Dublin (S. Dublin) is a serovar with high zoonotic potential, adapted to cattle, which causes both intestinal and systemic infections in the host[1]. Infections have significant impact on productivity and welfare in cattle herds, thus resulting in serious economic losses for the farmers[2]. Bacteria dissemination occurs mainly through feces, although spreading via aerosol, oculo-conjunctival route and milk secretion has been proved in cattle. Thus, contagion is possible either directly between animals or indirectly, due to its ability to survive for long time in the environment[3]. Ingestion of S. Dublin does not necessarily induce disease. In fact infection may result in chronic and subclinical carriers that may spread organisms continuously or intermittently in the environment not only through feces but also through milk and colostrum, thus constituting an important maintenance factor of the infection within the herd [4,5]. The pathogenesis of S. Dublin depends on several factors, such as virulence factors,

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infectious dose, passive transfer of specific immunoglobulins, immunity developed during previous infections, age, and physiological status of the host[6,7]. In particular, the development of a successful immune response is thus fundamental[8], even if several studies highlighted that metabolic pathologies, such as ketosis, might negatively impact on its stimulation[9,10]. Consequently, also the clinical presentation is affected, distinguishing an hyperacute, acute or chronic form[11,12]. Adults are generally affected by asymptomatic infection or subacute enteric form, and abortion could be the only clinical sign of infection. Calves may be affected more frequently than adults by the hyperacute form, characterized by sepsis and respiratory signs, with highest incidence between 4 and 28 days[13]. The severity of the disease is strictly connected both with rearing condition and with management[14]. Many calves often suddenly succumb 1 to 2 days after the onset of symptoms due to dehydration and generalized systemic distress, especially in case of no or inadequate drug treatment[15].

Generally, most recurrent clinical signs are apathy, anorexia, hyperthermia, reduced milk production (adults), respiratory distress (calves), mucosal pallor. These are followed by diarrhea, varying from greenish watery to fetid and yellowish, containing blood, mucus, fibrin, and necrotic shreds of the intestinal lining[15]. Meningoencephalitis, septicemic arthritis[16], dry gangrene in the extremities[17], urocystitis and ureteritis[18] have also been described.

S. Dublin is also an important serovar from human health perspective, as it demonstrates a high level of invasiveness in humans that could lead to severe disease or death[19]. Therefore, the occurrence of S. Dublin in dairy herds should be faced applying all the biosecurity measures needed to avoid the contamination of milk, the development of chronical infection in cattle and the spread of the disease inside and outside the farm. The aim of this study is to describe the application of a biosecurity protocol to control S. Dublin spread in a dairy farm of the Northeastern Italy.

CASE DESCRIPTION

One Italian herd of 385 Holstein-Friesian dairy cows, of which 210 in milk, was affected by an outbreak of S. Dublin. Only calves younger than 6 months showed clinical signs, in particular enteric syndrome, while deaths occurred only in animals younger than 30 days. In November 2020, a newborn calf died from enteric syndrome and a post-mortem examination was performed by the Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe). Necropsy finding included serious enteritis, peritonitis, and involvement of other organs such as liver, joints, lungs and pericardium. Specifically, icterus, pericarditis, fibrinous polyarthritis and foci of pulmonary congestion and hepatization were detected. Abomasitis, severe hepatosplenomegaly with hepatic degeneration and impressive meseraic lymphadenomegaly were also found. To investigate the etiology of the gross findings, intestine, joints, lymph nodes, spleen, pericardium, lungs and kidney were examined by standard bacteriological method and Minimum inhibitory concentration (MIC), revealing S. Dublin colistin-resistant as causative agent.

The case was reported to the Local Official Veterinary Service (LOVS), and a biosecurity management protocol in compliance with IZSVe was then applied.

PROTOCOL DESCRIPTION

The IZSVe protocol consisted in a farm analysis and epidemiological investigation and the collection and testing of screening samples to assess the spread of the disease and the absence of milk contamination.

Screening tests were performed both on fecal and milk samples. Feces were collected from 80 animals (dry-off cows and calves), in 7 times-points, from November 2020 to February 2021. 25 milk samples were collected from bulk tank milk (BTM) from November 2020 to April 2021. Furthermore, two environmental swabs were also collected from lactating and dry cows' boxes in November and December 2020.

Moreover, all lactating cows were tested by mean of a fecal swab 2 times, in February and March 2021: a total of 220 and 223 dairy cows were tested.

Due to the specific patterns of S. Dublin, the use of vaccination was included in the protocol.

Considering the difficulty to find an effective commercial vaccine against S. Dublin, an autogenous vaccine was prepared from the S. Dublin strain isolated from the farm by the IZS of Sardegna. The vaccine, consisting of a washed culture inactivated with 0.3% formalin and adjuvanted with 10% aluminum hydroxide, was administered to all the cattle of the farm with a first dose in February and a booster dose one month later.

A pre and post-vaccination screening was performed to assess the immunization of cows and the effectiveness of the protocol itself.

A total of 52 cattle randomly selected among cows and heifers were enrolled for the 3-time effectiveness sampling (T0, T1, T2), the first one day prior immunization, the second and the third 2 and 11 months later respectively, both on feces and serum, collecting blood samples from the coccygeal vein using a vacutainer system[20,10]. Due to animal culling only 44 animals completed the screening. To monitor the risk of milk contamination, weekly bacteriological control of BTM was performed from November 2020 to April 2021, for a total amount of 25 samples collected and tested.

ANALYTICAL METHODS

Salmonella spp. detection and typing:

Salmonella spp. detection on fecal samples, milk, animal tissue and environmental swab was performed according to ISO 6579:2017.

All the *Salmonella spp.* isolates were delivered to the National Reference Center and subtyped according to Kauffmann-White-Le Minor[21].

Antibodies detection:

To identify *Salmonella* serological antibodies an indirect ELISA commercial kit (PrioCHECK® *Salmonella* Ab bovine) was performed. Results were analyzed through the Percent positivity (PP). Cut off positivity was established with values of PP≥35%.

RESULTS

The epidemiological investigation highlighted as main biosecurity risk factors the purchase of adult animals, the practice of mountain grazing using promiscuous pastures and the absence

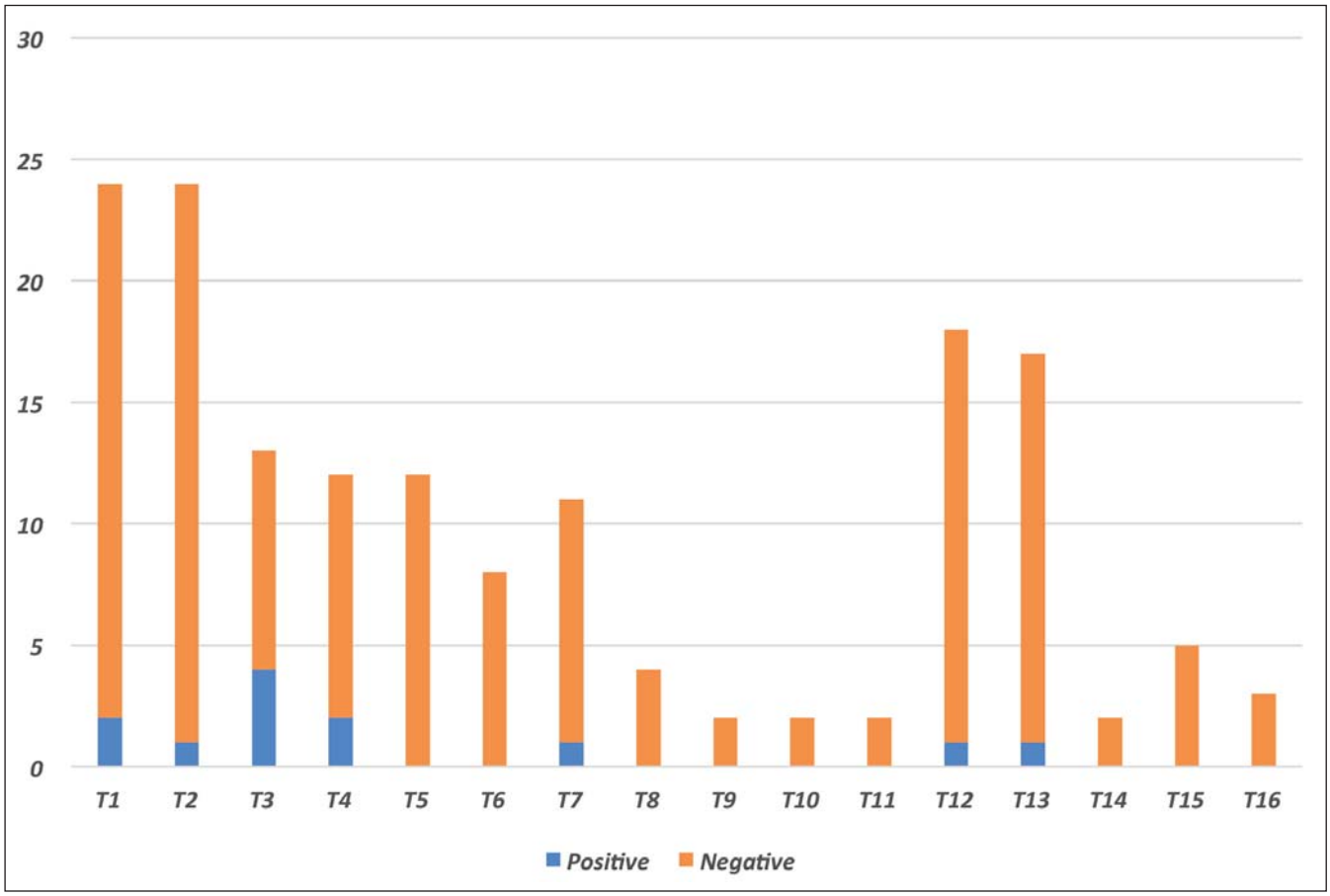

Figure 1 - Results of screening test on calves and cows.

of regular pests control plans. Factors that facilitated the spread of infection were overcrowding and poor environmental hygienic areas where both lactating and dry cows were housed, just as poor hygiene condition of the milking room and its equipment. Consequently, corrective measures were adopted, such as milking facility and routine hygiene improvement, implementation of an appropriate pest's control system, systematic cleaning, and disinfection of newborn calves' cages and of the equipment used to prepare and feed milk to calves. To reduce the risk of contamination, milk after collection was heat treated to be suitable for human consumption; to avoid the spread of infection to veal calves' farms, the control of all newborn calves was improved at least twice within 7 days, and the movement of positives calves was forbidden.

The screening test performed in the farms highlighted the presence of 6 positive calves with 2 calves that remained positive to 2 consecutive test, and 1 dry-off cow (Figure 1). All the fecal samples collected from the lactating cows in February and March tested negative for S. Dublin. All the environmental swabs collected in lactating and dry-off cows' pens tested positive for S. Dublin. Eventually 2 BTM samples out of 25 were contaminated with S. Dublin; both the samples were collected in November.

From the effectiveness evaluation sampling, serological examination identified 13/52 (25%), 49/49 (100%) and 32/44 (73%) positive animals at T0, T1 and T2 respectively. No fecal sample in all time-points was found positive. After vaccination only 1 milk sample turned out positive. Considering serological response of cows after vaccination, an initial strong increase in antibody titer at T1 and a subsequent decrease after 11 months (T2) were shown (Figure 2).

Serological analysis also showed that the presence of seropositivity before the first vaccination (T0) significantly affected the antibody response of cows (Figure 3).

DISCUSSION

The management protocol applied in this outbreak of S. Dublin reflects those commonly applied in case of positivity to S. Typhimurium, including the monophasic variant, S. Dublin and S. Enteritidis in dairy cows' herds. These serotypes were selected based on 3 factors: isolation rate in samples of bovine origin, public health relevance and zoonotic aspect.

Findings highlighted by the epidemiological investigation agree with several other studies, according to which the purchase of infected carrier animals could be one of the most frequent access ways of S. Dublin into farms[3,22,23]. Another key point is keeping small and stable groups of calves, avoiding mixing individuals with different immune and infectious status. Boxes of 2 or 4 calves would be optimal; on the contrary, groups of more than 8 calves would greatly increase the risk of S. Dublin outbreaks in young animals[2]. Subsequently, it has been shown that poorly clean calving boxes and overcrowding of calving animals have a negative impact on the spread of the disease. Proper management of calving boxes is therefore essential in the control of *Salmonella spp.* infection. Preventive actions should include removing calves from their mothers immediately after calving, setting up a separate box for each cow, maintaining high levels of cleanliness and hygiene in the environment and bedding, and allocating areas for the exclusive use of parturient cows[24,25]. An-

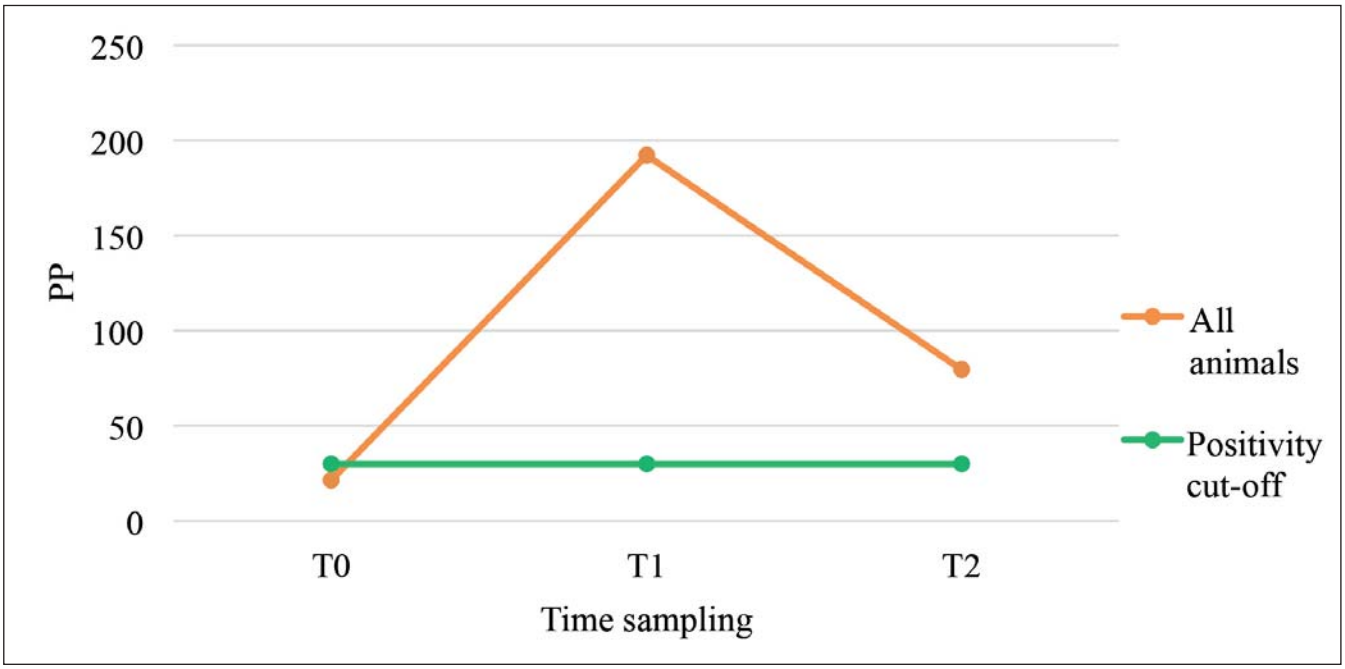

Figure 2 - Average serological Positivity Percentage (PP) trend in animals sampled with positivity cut-off of 30.

other important aspect is that chronically infected cows can eliminate S. Dublin through colostrum and milk. Several studies also showed that contamination of milk and colostrum, tends to increase through collection, storage and feeding processes, thus making cleanliness and hygiene of all the tools used for feeding calves and of barn staff crucial[24,26]. For this reason, it is essential to clean and sanitize feed and water distribution tools, environments and milking parlors with their respective equipment, as reported by numerous studies[4,24].

Analysis of the clinical and laboratory data showed that the infection occurred in both cows and calves, although probably with different prevalence between these groups.

The initial contamination of milk may be due to simultaneous presence of adult bovine animals excreting *Salmonella* and the hygienic deficiencies highlighted during milking and cleaning of the equipment used for this activity. Considering the positivity encountered in the housing areas of lactating cows, observed during the first sampling, we should hypothesize that the lack of detection of excretory cows is related to the sporadic excretion, which was probably present before the sampling was carried out.

The effectiveness evaluation highlighted the discrepancy between the serological test and the detection of S. Dublin in feces. In fact, at T0 some subjects tested positive to the serological test, without excretion of S. Dublin in the feces. The antibody positive outcome showed that animals had previous contact with the etiologic agent but without pathogen's spread. Considering the pathogenesis of S. Dublin infection, it cannot be excluded that some positive animals were chronic carriers[27]. The use of vaccination may improve the immune response and reduce the duration of fecal excretion, thereby helping to limit the spread of infection

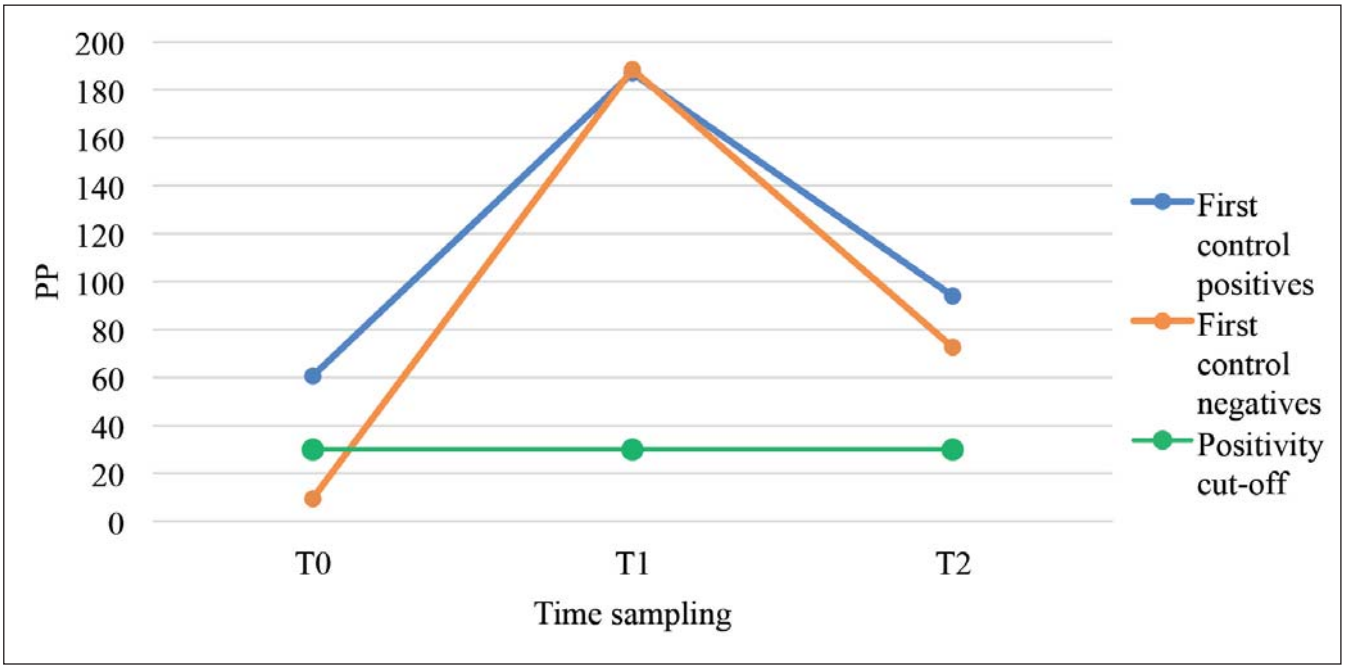

Figure 3 - Average Positivity Percentage (PP) trend in animals serologically positive or negative at first sample.

within the herd.

The initial strong increase in antibody titer at T1 and the subsequent decrease after 11 months (T2) highlight how the use of vaccination cannot be extemporaneous but must include annual booster to ensure adequate antibody titer to limit excretion phases in chronically infected animals.

Moreover, concerning serological positivity at T0, analysis showed that, after 11 months, these animals had higher values at the T2 than the initially seronegative animals. This difference may be related to a preexisting natural infection, which may induce a more sustained immune response in individuals. This event does not occur in all animals because, due to a malfunction of the cell-mediated immune response, chronic infections may develop[4]. Previous studies highlighted that animals with the highest risk of becoming active carriers are heifers aged between the first year of life and first calving, and cows infected close to the calving date itself. An increase in incidence was also noted in farms with low eliminator's prevalence[28].

Considering the negativity of all fecal bacteriological exams, this would suggest a possible positive effect of the vaccine in reducing the excretion of S. Dublin, considering that the direct prophylaxis measures applied also contributed to the biocontainment of the infection. However, it's not possible to quantify the vaccination's effectiveness, as the management protocol applied included both direct and indirect prophylaxis. On the other hand, based on the exams performed, control measures yielded good results in terms of reduction of clinical cases and circulation of the etiological agent.

CONCLUSIONS

Biosecurity and biocontainment measures adopted were effective in identifying eliminators and reducing environmental contamination. In addition, the adoption of a vaccination protocol has been a key factor in the management of the infection. Results of post-vaccination sampling, demonstrated how the integrated application of direct and indirect prophylaxis measures drastically reduced the presence of eliminator animals, leading to the extinction of the infection outbreak, thus constituting a possible model for the management of S. Dublin outbreaks in dairy herds even in complex farm situations.

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