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Salmonella enterica subsp. *diarizonae* Serotype 61:k:1:5:(7) a Host Adapted to Sheep

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Abstract

Salmonella genus is widely distributed in nature and causes a spectrum of diseases in man and animals. *Salmonella enterica* subsp. *diarizonae* serotype 61: k: 1,5, (7) (SED) is a host adapted to sheep and its presence as saprophytic bacteria in sheep has been described in different countries. Several studies performed in abattoirs reported the presence of SED in healthy sheep in the intestinal content and also in the respiratory tract. In addition, this microorganism has also been isolated from nostril and faecal samples in healthy live animals. For this reason, this microorganism is considered well adapted to sheep, behaving normally these animals as asymptomatic carriers. However, SED has also been reported causing health disorders such as chronic proliferative rhinitis in adult sheep, abortions, testicular lesions in rams or alimentary tract disorders in young animals. The zoonotic potential of this microorganism is also discussed.

Keywords: sheep, *Salmonella enterica* subsp. *diarizonae* serotype 61: k: 1,5, (7), respiratory tract, host-adapted, chronic proliferative rhinitis, zoonosis

1. Introduction

Salmonella spp. is a gram-negative, facultative intracellular anaerobe bacterium. It is a rod-shaped bacterium belonging to the family *Enterobacteriaceae* [1, 2]. It is also a ubiquitous and hardy bacterium that can survive several weeks in a dry environment and several months in water. Most serotypes are present in a wide range of hosts [3]. *Salmonella* spp. is a primary pathogen that is distributed worldwide. It can be found in several locations and is responsible for important disorders in both animals and humans. Certain serotypes are particularly relevant due to their zoonotic potential or because they cause relevant economic losses [4].

In animals, salmonellosis is presented in four major forms, such as enteritis, septicemia, abortion and asymptomatic carriage. In humans, salmonellosis includes several syndromes such as enteric fever, gastroenteritis, septicemia, focal infections and, in the case of some typhoidal strains, an asymptomatic carrier state [2]. Salmonellosis is endemic in most countries and causes substantial economic losses [5, 6]. *Salmonella* infections in farm animals, and their transmission to humans, have a substantial economic and social impact [7].

Three hundred serovars have been described in *Salmonella enterica* subsp. *diarizonae* (IIIb) group that was firstly isolated from reptiles [7]. Most of these serovars are isolated from coldblooded animals, and some of them can also affect humans. Reptiles carry *Salmonella* spp. as part of their intestinal flora, and shed the microorganism intermittently through their faeces. Infected faeces can contaminate, directly or indirectly, humans [8, 9].

Salmonella arizona serotype 61:K:1,5,7 (currently *Salmonella enterica* subsp. *diarizonae* serotype 61:k:1,5, (7): SED) was firstly identified in sheep in 1952 from carcasses of newborn lambs [10] and *Salmonella arizona* 61:k:1,5,7 was isolated for the first time in abortifacient material from sheep in England and Wales in 1976 [11, 12]. In 1999, in the UK, this specific serovar was responsible for all the identified incidents in sheep [13]. In addition, from 1998, SED became the most common serovar isolated from sheep in England, and in 1999 represented 45.7% of the total *Salmonella* incidents [14, 15]. Likewise, SED, along with *Salmonella abortusovis*, are the *salmonella* microorganisms more often isolated from ovine in Spain [4]. Moreover, the detection of SED has been increased in recent years.

SED is considered host-adapted to sheep, and it displays a very different epidemiological pattern than does the sheep-restricted *Salmonella enterica* serovar *abortusovis*. SED is able to produce both intestinal and extraintestinal infections with faecal, vaginal, and nasal colonisation, but mostly without clinical disease. These properties deviate from the classical characteristic of ubiquitous serovars. Therefore, the term “sheep-associated serovar” appears to be more appropriate for characterising it [16]. Interestingly, a bacterium apparently “host-adapted” to the digestive tract of some reptiles jumped between species and found the respiratory tract of sheep as a location for its saprophytic existence, mainly when it is not a common pathogen of the respiratory system neither in humans or animals.

2. *Salmonella enterica* subsp. *diarizonae* serotype 61:k:1:5:(7) host-adapted to sheep

Although *Salmonella* spp. has been mostly related to digestive and reproductive disorders, SED is a microorganism well adapted to the respiratory tract of sheep. The traditional association of bacteria of the genus *Salmonella* with digestive disorders has meant that, for many years, SED was mainly sought in the digestive system and its possible location in the respiratory tract was neglected [17, 18].

Several works reported the presence of SED in the intestinal content of healthy sheep. Thus, SED has been isolated from this location in the United Kingdom [12, 14, 19], Norway [20, 21], Switzerland [22–24], Iceland [25], Sweden [26], Canada [27], the United States [28, 29] or Spain [18]. All these studies suggest that sheep are a reservoir for this microorganism, thus being considered as a saprophyte microorganism of this specie. As mentioned above, in almost all these studies only intestinal content was analysed, then the percentages of isolation found were normally low. It is described a 1% in the UK, 2% in Iceland, 17.6% in Sweden and 11–43% in Switzerland [26]. The samples collected and analysed in these studies were either stool from live animals or intestinal content or gut sections in studies carried out in abattoirs. However, for the past two decades, it has been proven that SED is a common microorganism of the respiratory tract of sheep. Bonke et al. analysed the presence of SED in tonsils and faeces of healthy sheep at the abattoir, and they found 43% of positive adult animals in tonsils, while only a 2% of the faeces samples were positive and only in young animals [23].

A recent study was performed by our research group to investigate the prevalence of SED in nostrils and stool of healthy live sheep in Spain [18]. The data

collected in this study were analysed at two levels, animals and farms. The results showed that 45.3% of the animals were SED positive in nostrils or faeces, being the number of positive samples in nostrils higher than in faeces (38.5% vs. 22.5%). These data differ from those reported by Bonke et al. [23], that despite showing a high prevalence in tonsils all the adult animals analysed were negative in faeces. This was justified by the authors with poor conservation, and a small number of intestinal samples analysed. In our study, at farm level, nine of the ten analysed farms had at least one positive isolation of SED in one of the locations (nostrils or faeces). Further, all positive farms except one had SED isolations in both locations, nostrils and faeces, and in almost all positive farms sheep belonging to the youngest age ranges (0–2 and 2–4 years) accounted for more than 50% of positive isolates. The collective and individual prevalence in the studied region (Aragon, Spain) was estimated at 90% and 45.3%, respectively [18].

3. *Salmonella enterica subsp. diarizonae* serotype 61:k:1:5:(7) causing disease

The importance of this bacterium as a pathogen causing disease in sheep seems to have been increased in recent years, with the number of reports in international publications and conferences growing.

The first report of this microorganism as pathogen was related to abortifacient material from sheep of England and Wales in 1976 [12]. Several authors consider SED as a relevant abortive agent in sheep; however, a detailed analysis of different studies indicate that these bacteria appear along with other abortive microorganisms, suggesting a secondary role in these processes. Thus, Sojka et al. reported the presence of SED in nine abortion incidents, however in eight of them other abortifacient agents were also isolated, and on the ninth, SED was isolated in small numbers from placental cotyledons of only one of the two aborted lambs [12].

SED has also been associated with testicular lesions in rams [30, 31]. In both clinical descriptions, severe enlargement of the scrotal contents, fibrous adhesions between testicular layers, the coexistence of epididymal abscesses and testicular atrophy were described. This bacterium was isolated from the suppurative exudate in both cases, and the authors highlighted the importance of including this microorganism in the differential diagnosis of ovine genital infections.

Recently, SED was also associated with an outbreak of diarrhoea in lambs in Greece [32], where the presence of this microorganism was suggested as the cause of the digestive clinical signs in the lambs. Although SED is regularly isolated from faeces and tissues of the gastrointestinal tract from apparently healthy lambs and adult sheep [33], the pathogen has also been detected in faeces and tissues of lambs that had died from diarrhoea, thus gaining attention as a potential causative agent.

Although the previously mentioned incidents are infrequent health disorders associated with SED, chronic proliferative rhinitis (CPR) is a common disease that has been clearly related to this specific serovar of *Salmonella*. This disorder is precisely located in the upper tract of sheep where this microorganism is frequently isolated in healthy animals. CPR is a slow and progressive condition with an irreparable and poor prognosis for the untreated affected animals. It causes an inflammation of the ventral nasal turbinates causing very specific clinical signs that start with uni or bilateral thick seromucous nasal discharge together with snoring. These signs persist for several weeks or months and worsen, with almost complete nasal obstruction caused by the severe proliferation of the nasal mucosa of the turbinates in association with severe chronic inflammation, often visible at the nares [24, 34]. At this point, animals develop severe respiratory distress with striking mouth

breathing. The inadequate flow of air provides a better situation for opportunistic bacteria, and secondary pulmonary diseases can also be found. The affected animals are early removed from the flocks either because of their death or because of their premature health condition deterioration [17]. At *post-mortem* examination, the ventral turbinates are shown swollen and have a roughened surface [17]. The section of the turbinate shows a proliferative tissue and affected animals frequently have nasal deformation and deviation of the nasal septum [24]. Histopathological evaluation reveals a thickened nasal mucosa with multiple polypoid projections. These polyps are covered by hyperplastic respiratory epithelium. Gram staining reveals the presence of numerous gram-negative bacilli within many epithelial cells, and *Samonella* immunohistochemistry reveals intracellular dot or rod formations inside proliferating epithelial cells and macrophages [17, 24, 29]. Although CPR generally produces a proliferative inflammation of the ventral nasal turbinates, recently it was described for the first time the affection of the dorsal turbinate and ethmoidal areas in an adult sheep [35].

In the prevalence study of SED carried out in Spain by our research group [18], a significantly higher percentage of isolates of SED was found in the flocks with previous cases of CPR than in those in which the disease had never been diagnosed. This could suggest that the infection pressure in the farm might favour the occurrence of clinical cases of the disease, since, as concluded in the experimental infection carried out in 2017 [36], the simple presence of the bacteria in the nasal secretions is not enough to trigger clinical signs of the disease. It seems that other factors, yet to be discovered, are necessary for SED to pass through the epithelial cells of the nostrils and elicit the inflammatory reaction. Further studies will be necessary to unravel why this saprophytic bacterium of the high respiratory tract is able to cross the epithelial barrier causing severe inflammation in some animals.

4. Zoonotic potential of *Salmonella enterica subsp. diarizonae* serotype 61:k:1:5:(7)

Salmonellosis represents one of the most important zoonosis [16]. Salmonellosis in humans is generally contracted through the consumption of contaminated food of animal origin (mainly eggs, meat, poultry, and milk), although other foods, including green vegetables contaminated by manure, have been implicated in its transmission. *Salmonella* bacteria are prevalent in food animals such as poultry, pigs, and cattle and can pass through the entire food chain from animal feed, primary production, and all the way to households or food-service establishments and institutions [3]. Person-to-person transmission can also happen through the faecal-oral route. Human cases also occur where individuals have contact with infected animals, including pets. These infected animals often do not show signs of disease [3].

Salmonella enterica subsp. diarizonae is frequently isolated from the environment, cold-blooded animals, sheep and humans. However, only a few studies describe the isolation of this serovar from invasive human infections [37]. The rising popularity of exotic reptile as pets has led to an increase in the number of reptile-associated salmonellosis (RAS), considering it as an emerging zoonosis in humans [38]. All the zoonotic cases that have been described associated with SED were in persons that had some contact with reptiles, mostly as pets. *Salmonella enterica subsp. diarizonae* is the serovar most commonly isolated in patients with RAS. Young children and immunocompromised people seem to be especially prone to infections with reptile-associated *Salmonella* and often experience severe clinical courses, as it was described in different studies. Gastroenteritis in a neonate was presented, in which a regular contact of her mother with several pet reptiles was confirmed. The isolated serovar

isolated in this case was *S. enterica* subsp. *diarizonae* serotype 47:k:z35 [8]. Also, this serovar was reported on reptile-associated maxillary sinusitis in a Snake Handler in 2016 [38]. In the United States, more than one million cases of human *Salmonella* infection occur every year, and a great amount of these cases result from exposure to reptiles or amphibians [39]. In order to prevent RAS, the Centres for Disease Control and Prevention have recommended handwashing with soap and water after handling reptiles or reptile cages; these recommendations also stipulate that reptiles should not be kept near children and immunocompromised persons, and should not be allowed to roam freely throughout the home or living area [40, 41].

The zoonotic potential of *Salmonella enterica* supsp. *Diarizonae* serotype 61:k:1:5:(7) and the role of sheep in the transmission has been widely discussed [17, 26, 42], even though there have been no confirmed human cases associated with this specific serotype. In France, in 2008, a pseudo-outbreak in humans associated with SED was reported [43]. After a large number of SED positive samples from humans, trace-back investigations incriminated culture media containing contaminated sheep blood agar. None of the positive patients had suggestive symptoms of *Salmonella* infection. All samples had been taken during routine screening and SED was isolated from different body sites, including nine from usually sterile sites. The unusual clinical presentation and unusual serotype of *Salmonella* led to the suspicion that the origin of the contamination might be linked to the laboratory processing of the samples. After some investigations, they revealed that nine of the ten isolates had grown on sheep blood agar from the same manufacturer and that the batch number was similar for three cases. The manufacturer confirmed that the samples of the blood agar were positive to SED, owing to contaminated sheep blood [43].

On the other hand, and to emphasise that the discussion of zoonotic potential of this bacterium is still on the table, despite the high prevalence of SED in countries where other zoonotic salmonellas are under control, such as Sweden, Norway or Finland [18, 26], there have been no cases of human salmonellosis associated with this microorganism. As scientific opinions and evaluation of on-farm control measures performed in Sweden concluded that the impact of sheep associated *S. enterica* subsp. *diarizonae* on human health was very low, Swedish authorities decided to make an exemption for *S. enterica* subsp. *diarizonae* in sheep in the current *Salmonella* control measures and, in Norway, it was concluded that the impact of *S. enterica* subsp. *diarizonae* on human health appeared to be marginal [18, 26].

5. Conclusions

Salmonella enterica supsp. *Diarizonae* serotype 61:k:1:5:(7) is a host-adapted to sheep, being commonly isolated from upper respiratory tract of healthy sheep. This microorganism has been probably under-reported because traditionally it has been sought in the digestive tract of sheep and lambs when its more frequent isolation is from the upper respiratory tract. Some recent studies clarified the prevalence of SED in the nasal mucosa of healthy animals in different flocks.

Chronic proliferative rhinitis is an upper respiratory tract disorder clearly related to SED. However, the kinetics of the infection is not entirely understood, and further studies will be necessary to uncover why this saprophytic bacterium of the high respiratory tract is able to cross the epithelial barrier causing severe inflammation in some animals. In recent years, the number of CPR reports has been increased, what could mean that the knowledge and a proper description of the disease can lead to identifying new cases. Finally, it is essential to highlight that there are still some concerns about the zoonotic potential of this bacterium and the relevance of sheep as a reservoir of the infection.

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