Chronic proliferative rhinitis in sheep: an update

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ABSTRACT

Chronic proliferative rhinitis (CPR) is a slow and progressive upper respiratory tract disease of sheep with a poor prognosis for affected animals. It causes a proliferative inflammation of the ventral nasal turbinates with uni or bilateral affection that may totally obstruct the nasal cavity. *Salmonella enterica* subspecies *diarizonae* serovar 61:k:1,5,(7) has been associated with the disease. This microorganism, host-adapted of tonsils and nasal cavity of sheep, has been isolated in pure culture from the nasal cavity in all reported cases of CPR. However, its etiopathogenesis, mechanism of transmission and its involvement with the disease are still unknown. The present article focuses on describing the current knowledge about this disease gathering the published information and offering some new data on the latest research carried out on chronic proliferative rhinitis in sheep.

Keywords: sheep, chronic proliferative rhinitis, *Salmonella enterica* subsp. *diarizonae* serovar 61:k:1:5:(7), treatment, differential diagnosis.

1. Introduction

Respiratory diseases are common and relevant in all the major sheep-rearing countries. The intensification of this sector, is not followed by the improvement of the facilities and has encouraged the presentation of new pathologies that were less relevant in the last years (Lacasta et al., 2008; EFSA AHAW Panel, 2014; González et al., 2016).

Currently, respiratory problems are mostly associated with ovine respiratory complex (ORC) in young animals. However, there are other less frequent respiratory disorders affecting adult animals that also have a direct impact on the productivity in sheep production. In that sense, the influence and importance of upper respiratory tract pathologies is being increased in recent years (Brogden, 1998; Figueras, 2017; Lacasta et al., 2017). The spectrum of nasal pathologies in sheep is quite extensive. Some of these pathologies are well known and has been studied deeply such as oestrosis (Gracia et al., 2010), or enzootic nasal adenocarcinoma (ENA) (De las Heras et al., 2003), however, others are still under research as it is the case of chronic proliferative rhinitis (CPR). Furthermore, different upper respiratory tract diseases have been described in Australia or America, but not yet in Europe, as conidiobolomicosis or rhinofacial pitiosis (Carrigan et al., 1992; Portela et al., 2010).

Chronic upper respiratory inflammatory diseases in sheep are mostly associated with *Oestrus ovis* in Spain (Gracia et al., 2006), and it is considered the most important and prevalent rhinitis in hot and dry countries (Alem et al., 2010; Gracia et al., 2010). Larvae of *Oestrus ovis* are obligated parasites in the nasal passages and sinuses of small ruminants, therefore, this is a very common disorder in regions of extensive breeding of sheep (Lucientes et al., 1998). The other relevant upper respiratory tract disease is the enzootic nasal adenocarcinoma of sheep and goats that is a neoplastic transformation of nasal glands of the ethmoidal area (De las Heras et al., 2003; Walsh et al., 2013). As the tumor grows, the animals present cranial deformation and exophthalmos.

CPR associated with *Salmonella enterica* subsp. *diarizonae* serovar 61:k:1,5,(7) (SED), that was originally described for the first time in USA in 1992, at that time as *Salmonella arizonae* (Meehan et al., 1992), is becoming currently a relatively common disease in Spanish sheep farms (Lacasta et al., 2012; Lacasta et al., 2017). In the last year, Stokar-Regenscheit et al., (2017) and Wolf and Schefers, (2017) referred cases of CPR in Texel breed sheep in Switzerland and United States. This specific serovar of *Salmonella* has been isolated as a sole microorganism in pure culture from nasal cavity in all the reported clinical cases of CPR. The implication of this agent in various diseases in sheep and its role as a potential zoonosis are discussed (Lacasta et al., 2012; Soren et al., 2015; Methner and Moog, 2018). There are some concerns that these bacteria may have zoonotic potential (Horvath et al., 2016). Human pathologies associated with these bacteria are rare and generally occur in individuals with underlying disease or in individuals who have handled reptiles, where these bacteria are common (Schröter et al., 2004), so far there have been no human cases related to sheep.

2. *Salmonella enterica* subsp. *diarizonae* serovar 61:k:1,5,(7)

Salmonella is a Gram-negative, facultative intracellular anaerobe. It is a rod-shaped bacterium belonging to the family *Enterobacteriaceae* (Coburn et al., 2007; Agbaje et al., 2011).

Subspecies IIIa and IIIb, also known as *S. enterica* subsp. *arizonae* and *S. enterica* subsp. *diarizonae,* were first identified in sheep in 1952, from carcasses of newborn lambs (Ryff and Browne, 1952) and *S. arizona* 61:k:1,5,7 (currently *S. enterica* subsp. *diarizonae* serovar 61:k:1,5,(7)) was first identified in abortion material from sheep in England and Wales in 1976 (Long et al., 1978; Sojka et al., 1983). Although there are approximately 300 serovars in the diphasic arizona diarizonae subspecies

(Old, 1992), in the UK a single serovar, *Salmonella enterica* subspecies *diarizonae* serovar 61:k:1,5,(7) was responsible for all the identified incidents in sheep (Anon, 1999). From 1998, SED became the most common serovar isolated from sheep in England, and in 1999 represented 45.7% of the total *Salmonella* incidents (Davies et al., 2001; Milnes et al., 2008). Moreover, this serovar has been detected in sheep in different countries as Switzerland (Zweifel et al., 2004; Bonke et al., 2012; Stokar-Regenscheit et al., 2017), Iceland (Hjartardottir et al., 2002), Sweden (Soren et al., 2015), Norway (Alvseike and Skjerve, 2002), Canada (Pritchard, 1990) United States (Dargatz et al., 2015; Wolf and Schefers, 2017) or Brazil (Celeghini et al., 2013), suggesting that sheep are a reservoir for this microorganism. In Spain, SED is the most common isolated after *Salmonella enterica* subsp. *enterica* serovar *abortusovis* (according to unpublished data of the Agroalimentary Laboratory of Aragón; J.P. Bueso personal communication) and it has been described causing unilateral orchitis and epididymitis in a ram (Ferreras et al., 2007; Celeghini et al., 2013), and CPR in adult sheep (Lacasta et al., 2012). SED has also been associated with ovine abortions/stillbirths and alimentary tract disturbances (Long et al., 1978; Brogden et al., 1994; Davies et al., 2001; Chatzopoulus et al., 2016). However, isolates from faecal samples failure to reproduce enteric disease in lambs (Harp et al., 1981) and oral administration to pregnant sheep did not induce any pathology (Hannam et al., 1986). It seems that the majority of sheep, adult and juvenile, are asymptomatic carriers.

3. Clinical and pathological findings

CPR is an upper respiratory tract disease of sheep. It is slow and progressive with an irreparable and poor prognosis for the untreated affected animals. It causes an inflammation of the ventral nasal turbinates that produces very specific symptoms. The clinical signs 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 inflamation, often visible at the nares (Fig. 1A) (Stokar-Regenscheit et al., 2017). At this point, animals develop severe respiratory distress with striking mouth breathing (Lacasta et al., 2012) (Fig. 1B). The inadequate flow of air provides a better situation for opportunistic bacteria and secondary pulmonary diseases can be also found. The animals are early removed from the flocks either because of their death or because of their premature health condition deterioration. In the physical examination, the regional lymph nodes could be enlarged, and from swabs collected from the nasal cavity of sheep with clinical signs SED is always isolated.

As it was mentioned here above, CPR produces a proliferative inflammation of the ventral nasal turbinates, while the dorsal and ethmoidal conchae show minor changes and the affection can be uni or bilateral. At post-mortem examination, the ventral turbinates are shown swollen and have a roughened surface (Lacasta et al., 2012). The section of the turbinate shows a proliferative tissue that is usually composed of multiple small white or yellow polypoid structures covered by mucus, although only a thickening of the mucosa can be observed (Fig. 2). The affected animals frequently have nasal deformation and deviation of the nasal septum (Stokar-Regenscheit et al., 2017).

Histopathological evaluation reveals a thickened nasal mucosa with multiple polypoid projections. These polyps are covered by hyperplasic respiratory epithelium or an epithelium composed of several layers of disorganized epithelial cells. These cells have elongated eosinophilic cytoplasm or vacuoles fill with amphophilic and ill-defined rods or dots. Gram staining reveals the presence of numerous gram-negative bacilli within many epithelial cells corresponding to the dots or rods observed on HE staining. Nuclei are generally rounded with peripherally condensed chromatin and some nuclei showing degenerative changes. Groups of neutrophils invade basal or apical portions of the epithelium focally. Eosinophilic amorphous material mixed with cell debris cover the proliferating epithelium. The lamina propria is expanded and densely infiltrated by plasma cells and a few macrophages. Nasal glands are hyperplasic, but preserve normal structure and their lumina are dilated to a variable degree by secretion (Fig. 3). Immunohistochemistry reveals intracellular dot or rod formations of approximately 1 μm in diameter inside proliferating epithelial cells. These are also found inside some interstitial macrophages (Lacasta et al., 2012; Stokar-Regenscheit et al., 2017; Wolf and Schefers, 2017).

4. Differential diagnosis and imaging techniques

A proper diagnosis should be based on the clinical examination and pathological findings found at necropsy. Furthermore, it is necessary to have a knowledge of the other diseases affecting the upper respiratory tract in order to perform an appropriate diagnosis.

There are many different diseases affecting the upper respiratory tract in sheep that could confuse the diagnosis, such as oestrosis, enzootic nasal adenocarcinoma or other more exotic diseases in Europe as conidiobolomiscosis, common in America or Australia. In areas with semiarid climatic conditions, oestrosis is the most important rhinitis (Abo-Shehada et al., 2000; Gracia et al., 2010). The main clinical difference between oestrosis and CPR is the prevalence. The oestrosis is a collective disease with a high prevalence, however, CPR is usually an individual pathology, affecting few animals in the flock at the same time (Figueras, 2017). Furthermore, in oestrosis the clinical signs have a seasonal variation, being more severe during hot and dry periods, because in cold months the larvae can overwinter in a diapause state. According to the clinical signs, in both cases affected animals present nasal discharge, but the mucus appearance is different. The animals affected with oestrosis present mucous or purulent nasal secretion or even haemorrhagic due to the erosion of the larvae in the nasal cavity, nevertheless, CPR causes serous or seromucous nasal discharge. The respiratory distress is taken place in both cases, but the CPR goes together with a very characteristic snoring, that allows a quick and close diagnosis (Alem et al. 2010; Gracia et al., 2010; Rubira et al., 2017).

The enzootic nasal adenocarcinoma (ENA) is originated from the ethmoidal conchae of the nasal cavity (Walsh et al., 2013). It is also an individual pathology with low prevalence, however, clinically, the affected animals show stertorous breathing and profuse serous nasal discharge, that washes the nostrils. As the disease progresses, the animals present facial swelling, and exophthalmos that facilitates its diagnosis. As CPR, this disease has a poor prognosis for the affected animals (De las Heras et al., 2003).

Finally, we would like to mention in the differential diagnosis the ovine pulmonary adenocarcinoma (OPA), because, in spite of being a lower respiratory tract disease, its clinical signs may be confusing. In OPA, there is usually a thin mucus discharge from the nostrils, and if the head is lowered, a copious frothy exudate may pour from the nares, however, moist crackers may be heard on auscultation, so we could identify that it is a lung disorder (Borobia et al., 2016; Scott and Cousens, 2018). Although CPR is a disease of the upper respiratory tract, secondary problems can be also found in the lung due to inadequate flow of air, which provide a better situation for the opportunistic bacteria.

Nevertheless, for the correct diagnosis of this disease in live animals, imaging techniques can be very useful. Thermography, ultrasonography, radiography and computed tomography (CT) have been used to support de clinical diagnosis of the CPR and they are useful tools to develop the understanding of the disease.

Infrared thermography is a non-invasive technique that allows the remote measurement of the surface temperature of an object or animal. A thermal imaging camera captures and records the measurement and creates a thermal image. It provides colour images, where each colour corresponds to a specified temperature according to a reference scale. Although there are still no studies with reference values, in the authors' experience, sheep with healthy nasal cavity have a temperature between 18 and 23ºC, depending of the temperature of the environment that refrigerates the cavity. Already animals affected by rhinitis present temperatures greater than 28ºC. These measurements can be evaluated to establish their physiological or pathological meaning, detecting inflammation or subclinical signs, until the disease becomes evident (Holzer et al., 2010, Luzi et al., 2013). In the last years, thermal images were taken of CPR affected sheep at the Veterinary Faculty of Zaragoza, Spain. As it is showed in Fig. 4A, animals with a healthy nasal cavity have a correct pass of air throughout the nostrils, therefore they are completely refrigerated and the colour of the area is blue. In CPR affected animals, the thermal camera detects warmer temperatures in the nostril area. This temperature match with the inflammation caused by the disease and with the incapacity of a correct nasal cavity refrigeration due to the inflammatory and proliferative tissue that grows from the ventral conchae (Fig. 4B and 4C) (Rubira et al., 2017).

In the author’s experience, ultrasound imaging of animals with CPR can be performed and compared with those without nasal disorders. They have been carried out by positioning the probe through the space located between the free piece of the nasal bone and the maxillary bone, giving direct access to the turbinate’s projection. On the ultrasonographic images of animals without pathology, there is only a hypoechoic area, however, in the images performed from CPR affected animals, it seems to be some echogenic or hyperechoic tissue, which could match with the proliferative tissue that the disease produces (Fig. 5). Nevertheless, this image would be similar in other inflammatory rhinitis, so this imaging technique would not be the technique of election for the properly diagnosis (Rubira et al., 2017). Moreover, ultrasound images of this area are not easy to interpret, because a correct image interpretation requires profound knowledge of animal anatomy and physiology, as well as of the abilities of gas, fluids, tissues and organs to reflect the ultrasound wave (Crilly et al., 2017; Meinecke-Tillmann, 2017). Although with experience and knowledge of the technique, ultrasound equipment is readily portable and can be used under almost all farm conditions (Scott, 2017).

Radiography and CT are both techniques based on the emission of X-ray. Radiography uses X-radiation to penetrate a physical object to view its internal structure in 2D projections, while computed tomography is an imaging technique that creates cross-sectional images of a 3D object from 2D X-ray images taken from various positions (Kruttschnitt and Lueth, 2018). These two techniques allow the location of the proliferative tissue inside the nasal cavity, and its identification as a proliferative inflammation of the ventral nasal turbinates. Although both imaging techniques are not suitable in sheep veterinary practice in field conditions, the use of these techniques in the study of CPR with research purposes has provided exclusive images of the disease and it has improved the description and characterization of CPR, enabling the confirmation of the extent of the injured area. The affection of the ventral conchae can be seen with two radiographic lateral projections, one of each side (Fig. 6). The computed tomography or CT gives cross-sections of the skull that enables the extent of the disease (Fig. 7).

5. Treatment and prevention

There is scarce information about the etiopathogenesis and the mechanism of action of SED in the development of CPR. It is defined as a chronic disease with a poor prognosis for the affected animals (Meehan et al., 1992; Lacasta et al., 2012; Stokar-Regenscheit et al., 2017; Wof and Schefers, 2017), however, no treatments have been described for the sheep species. During the last three years, different treatments for clinical cases of CPR referred to the Ruminant Clinical Service of the Veterinary Faculty of Zaragoza, Spain, had been attempted (Table 1). As a result of the absence of information, several molecules were used according to the antibiotic susceptibility test of *Salmonella enterica* subspecies *diarizonae* serovar 61:k:1,5,(7), performed by the Kirby-Bauer Disk Diffusion Susceptibility Test (Table 2).

At the beginning, spiramycin was used by systemic route and no satisfactory results were obtained because, after treatment, the affected animals still presented clinical symptoms of the disease and positive isolation of the microorganism. After this attempt, a protocol for each treatment was established. For the microbiological evaluation, samples of the nasal cavity were collected with nasal swabs, before and after each treatment, in order to verify if SED was still inside of the nasal cavity or not. The samples were sent to the Agroalimentary Laboratory of Aragón for microbiological analysis. Samples were cultured into Buffered Peptone Water (APT) and *Salmonella Shigella* Agar (SS). Culture media were incubated at 37ºC for 24-48 hours. When no colonies of presumptive *Salmonella* were observed on SS after 24 hours of incubation, 1 ml of APT was transferred into a Muller Kauffmann Tetrathionate-noboviocin broth (MKTTn), incubated at 37ºC for 24 hours and spread onto the surface of an Xylose Lysine Deoxycholate Agar (XLD) and SS. Colonies resembling *Salmonella* were chosen and identified by conventional biochemical test: Tripe Sugar Iron agar (TSI), Lysine decarboxilase, Ornithine decarboxilase, Arginine dihydrolase, Christensen´s Urea, Voges-Proskauer and the Beta-galactosidase test. At the same time, to evaluate the clinical signs, thermal images were captured before and after each treatment. A decrease in temperature of the nasal area was related to an improvement of the clinical signs.

Other treatments were performed with different molecules according to the antibiotic susceptibility test. In these cases, the administration was through the nasoincisive notch to inoculate the substance directly in the ventral conchae (Fig. 8). There were no significant results in none of the treatments. At the end of each treatment a slight clinical improvement was noticed, but, usually, a few days later the animals reverted the symptoms. A complete resolution of the symptoms was not achieved and the attempt at treating these animals to eliminate SED was unsuccessful in every treatment.

Nevertheless, although the last and longer treatment with marbofloxacin (fluorquinolone) was not microbiologically successful, it appears that the animals recovered clinically better. Fluorquinolones have been described in long treatments against *Salmonella typhi* in humans (Jurado et al., 2010) and in a case of reptile-associated maxillary sinusitis due to *Salmonella enterica* subspecies *diarizonae* serovar 47:kz35 in a snake handler (Horvath et al., 2016). Moreover, fluorquinolones have great potency against Gram-negative bacteria, such as *Salmonella* spp. They are also concentrated intracellularly in macrophages and polymorphonuclear leukocytes, thereby enhancing activity against intracellular pathogens (Carlier, 1990). CPR is a disease with a chronic course; apparently caused by an intracellular microorganism, for this reason a long-time treatment with an intracellular-activity drug could be an option. However, more studies are needed to finally understand the pathogenesis of the disease and subsequently, to find an appropriate treatment.

6. Conclusions

There are still some concerns about this bacterium and its zoonotic potential. Generally, human pathologies associated with these bacteria occur in individuals with underlying diseases. However, the kinetics of the infection are not completely understood. Further studies may clarify the prevalence of SED 61:k:1,5,(7) in different flocks and the persistence in the nasal mucosa of sheep. Imaging techniques have been very useful to approach and clarify the diagnosis, although they must be together with a thorough clinical examination and sample collection. Furthermore, the treatments that have been carried out so far, do not reach the elimination of the bacterium. Therefore, more studies are being developed to achieve a curative therapy for the affected animals.

Conflict of interest statement

The authors have nothing to disclose.

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Figure legends

Figure 1. Animals affected with chronic proliferative rhinitis. **A**: Proliferating tissue appearing through the nostrils. **B**: Severe respiratory distress with striking mouth breathing.

Figure 2. Pathological finding of chronic proliferative rhinitis. **A**: Inflammation of the ventral nasal turbinate. **B**: Proliferative tissue composed of multiple small white or yellow polypoid structures covered by mucus.

Figure 3. A: Histological view of the thickened nasal mucosa with multiple polypoid projections. HE. Bar, 100 µm (Lacasta et al., 2012). B: Presence of numerous gram-negative bacilli inside of epithelial cells (black arrows) HE. Bar, 25 µm (Lacasta et al., 2012).

Figure 4: Thermal images from three sheep´s face (frontal view). **A**: Physiological perspective, the nasal cavity of this animal is completely refrigerated; therefore, the colour is blue meaning cooler temperature (Sp1 and Sp2 show a low temperature, 18.6º). **B**: Pathological perspective, animal affected with unilateral CPR. **C**: Pathological perspective, case of an animal with bilateral affection (Sp1 and Sp2 show higher temperature as in physiological conditions, 30.7º and 31.0º).

Figure 5. A: Ultrasound image of a sheep with a homogeneous hypoechoic tissue (without pathology) in the nasal cavity. **B**: Ultrasound image of a sheep with heterogeneous echogenic tissue suggestive of chronic proliferative rhinitis (3.5 MHz convex transducer, depth 7.5 to 8.6 cm).

Figure 6. Radiographic image from two sheep's face (lateral view) A: Physiological perspective. B: Pathological perspective, radiographic image of an animal affected by CPR. There is a mass located on the ventral conchae (yellow circle).

Figure 7. **A**: Positioning of the animal during the CT. **B**: Physiological perspective, CT of an animal without pathology on the nasal cavity. **C**: CT of an animal with bilateral affection due to CPR. It can be seen that the extent of the damage is different in each side, being larger on the left side (red arrows).

Figure 8. Skull of a mutton (Popesko, 1998) Red arrow: nasoincisive notch. Dotted line: direction of the local treatment's inoculations.

Table 1. Details of the different therapeutic protocols developed during four years; active substance, dosage, administration route and treatment period in sheep affected by CPR are included.

Year	Active substance	Dosage	Administration route	Treatment period
2014	Spiramycin	1ml/20kg BW /48h	Intramuscular	3 inoculations
2014	Spiramycin	1ml/conchae/48h	Local	3 inoculations
2015	Oxytetracyclin	1ml/conchae/48h	Local	5 inoculations
2016	Florfenicol	1ml/conchae/48h	Local	5 inoculations
2016	Florfenicol	1ml/conchae/24h 2ml/15kg BW	Local Subcutaneous	3 inoculations 1 single inoculation
2017	Marbofloxacin	1ml/50kg BW /24h	Intramuscular	5 inoculations
2017	Marbofloxacin	1ml/50kg BW /24h	Intramuscular	15 inoculations

Table 2. *Salmonella enterica* subespecies *diarizonae* serovar 61:k:1,5,(7) antibiotic susceptibility test results.

Susceptibility test	SED
Lincomicin	S
Erythromycin	R
Oxytetracycline	S
Florfenicol	S
Sulfami.-Trime.	S
Colistin	S
Tylosin	R
Neomycin	R
Marbofloxacin	S