Pathology and aetiology of Ovine Respiratory Complex in lambs

J.M. González[a,b *], T. Navarro[a], D. Lacasta[a], J.J. Ramos[a], L.M. Ferrer[a], M. de las Heras[a]

[a] *Animal Pathology Department. Instituto Agroalimentario de Aragón-IA2 (Universidad de Zaragoza-CITA). Veterinary Faculty of Zaragoza. C/Miguel Servet 177. 50013 Zaragoza. Spain.* [b] *Gabinete Técnico Veterinario, SL, 50014 Zaragoza. Spain.*

* Corresponding author; e-mail: jmgsovino@gmail.com

ABSTRACT

The ovine respiratory complex (ORC) in lambs presents three main clinical presentations: hyperacute, acute and chronic. The hyperacute form presents vascular damage and lesions in the upper respiratory tract and regional lymph nodes, which can be confused with another type of septicaemia. The chronic forms are the most frequent presentations of ORC, although they are mostly abattoir findings and the lesions are located in the lung and in the pleura. The acute form is the most common causing mortality and shares lesions with the other two forms, vascular damage, lesions in upper respiratory tract and consolidated lung injury. These lesion patterns determine the type of sampling for microbiological analyses. Bacteria are the most important agents of ORC in lambs and the cultures with the presence of *Pasteurellaceae* and/or *Mycoplasmas* spp. represent more than 80% of the total. However, these bacteria are part of the microbiota of the respiratory system and, moreover, these cultures are usually mixed. The most frequently isolated agents depend on the type of lesions and the age of the affected animal. In lactating lambs, *Manneheimia haemolytica* is the most common for both hyperacute and acute forms, whereas *Pasteurella multocida* is in chronic forms. During the fattening period, the most common isolated cultures are *Bibersteinia trehalosi*, *Mannheimia haemolytica* and *Pasteurella multocida* for hyperacute, acute and chronic forms, respectively. However, this can only be used as a guide for a correct sampling. The results obtained from the microbiological analysis are necessary to implement treatment or specific preventive plans.

The present manuscript is completed by other four articles included in this journal, dealing all of them with different approaches of ovine respiratory complex in lambs.

Keywords: ovine respiratory complex, *Mannheimia haemolytica*, *Bibersteinia trehalosi*, *Pasteurella multocida*, *Mycoplasmas*, sheep.

1. Introduction.

In the previous articles, three mean types of presentations of the respiratory disorders in lambs have been established and it has been commented how these patterns can be more frequently associated with the presence of an etiological agent (Navarro et al., 2019). In this article these clinical presentations will be deeply defined and practical recommendations for the sampling of each of these patterns will be made. Finally, the most important bacterial aetiologies found in these presentations will be shown. All this will be carried out through an eminently practical approach that allows the veterinarian to face the

diagnosis, implement a treatment and design specific prevention plans against ovine respiratory complex (ORC).

2. Lesion patterns

Before defining the most characteristic lesions of each one of these presentations observed in the ORC, we must remember that, as it has been defined in the previous articles of this special issue various diseases are grouped under this denomination. Each of these diseases is characterized by a series of lesions that we divide into different lesion patterns. These lesion patterns have been established according to the location of the most important lesions (upper and lower respiratory tract), the damage of the lymphoid tissue associated to the respiratory system, the type of pneumonia and the degree of chronicity of these lesions. However, all these patterns can be present at the same time in the same flock and even in the same lamb.

2.1. Hyperacute clinical form, septicemic lesions.

The main characteristic of this type of presentation of ORC is the sudden death of lambs that usually present a good body condition in which hardly any presence of clinical signs is observed. The lesions are located mainly in the upper respiratory airways and the presence of haemorrhages is very frequent. Sometimes, it is possible to observe the exit of haemorrhagic fluids through the nostrils. In the lung, the lesions are usually diffuse with predominance of vascular damage, in which haemorrhages and alveolar oedema are remarkable (De las Heras and Ferrer, 2001).

The lymphoid tissue of the whole respiratory system is enlarged, haemorrhagic and even necrotic (Figure 1). The damage is especially intense in the retropharyngeal lymph nodes and tonsils (Gilmour and Gilmour, 1985). It should be remembered that tonsils in sheep are composed by a total of six areas that are located around the epiglottis and proximal part of the oesophagus (Casteleyn et al., 2011). These patterns are often associated with situations in which lambs suffer high stress, such as weaning and heat stress, which reduces the immune response allowing the growth of bacteria and / or the production of large amounts of toxins. The necrosis of the lymphoid tissue may be the main macroscopic feature that allows us to differentiate these lesion patterns, that have traditionally been associated with septicemic pasteurellosis, from other septicemic or toxic processes such as clostridial diseasess (Gilmour and Gilmour, 1985). This sort of ORC presents the greatest degree of complication in anatomopathological and microbiological diagnosis (Navarro et al., 2019). Choosing the correct sampling organ and interpretation of microbiological analyses will be especially important in the diagnosis of this type of cases.

2.2. Chronic pattern.

In contrast to the septicemic lesions, chronic patterns are characterized by lesions in the lower respiratory tract and a low presence of vascular damage. They are the most common form of ORC in lambs (Navarro et al., 2019) although, in most of the cases, they do not cause the death of the animal and are just findings at the necropsy or at the slaughterhouse (Hervas et al., 1996; Goodwin et al., 2004; McRae et al., 2016). However, these lesions cause decreases in production indicators (growths, feed efficiency and yield carcass) and a deterioration of product quality (less fat in the carcass and less lifespan of meat) (Gonzalez et al., 2016). Depending on the severity of the lesions, the lambs may show severe symptoms for a long period of time with a relevant loss of body condition or just arrive at the abattoir without any clinical signs.

Animals affected by a chronic ORC present consolidated lung lesions that may be associated with atypical pneumonia or be the result of the evolution of an acute injury. Several types of pneumonias can be observed in these lambs, suppurative bronchopneumonia, with or without interstitial pneumonia, are the most common (López and Martison, 2017). These pneumonias usually affect the apical lobes maintaining a lobular distribution and are characterized by the large presence of inflammatory cells and purulent or mucopurulent exudate in the bronchi, bronchioles and alveoli. Sometimes these injuries suffer complications such as pleural adhesions, abscesses, renal amyloidosis, associated with the presence of numerous abscesses, or fibrinous / fibrous pericarditis (Donachie, 2000; Lindström et al., 2018). When this occurs, the risk of death increases, and productive declines are more important.

2.3. Acute ovine respiratory complex pattern.

Between the chronic and hyperacute patterns are located the acute patterns that are very variable depending on how close they are to each of them. Hence, acute patterns share characteristics with the other two patterns, so upper respiratory tract lesions with vascular damage are observed and consolidated pulmonary lesions. The proximity to chronic or septicemic patterns depends on factors such as the immune system status, the agent involved or the presence of toxins. These animals show severe clinical signs and usually die in two or three days (Bell, 2008). These lesion forms are frequently found at the necropsy of lambs in both farm and feedlot (Navarro et al., 2019). This pattern has traditionally been associated with pasteurellosis, in most of the cases, however, many other agents have been isolated in pure and massive cultures from these lesions.

The most characteristic pneumonic lesion found in this pattern is fibrinous bronchopneumonia, which is often accompanied by fibrin in the pleura (De las Heras and Ferrer, 2001; Benavides et al., 2015). Histologically, it is characterized by the presence of exudate, haemorrhages, inflammatory cells, oat-cells, and necrosis (Donachie, 2000). In addition, lesions in the upper respiratory tract can be

observed altogether with lung lesions, haemorrhages in the nasal cavity and tonsillar necrosis. Frequently, septicemic lesions can also be accompanied by haemorrhages in heart and brain.

3. Sampling for microbiological examination

Bacteria are the most important microorganism among the infectious agents associated with the presence of ORC compatible lesions. The knowledge of the agent involved in the cases of ORC is necessary to carry out preventive measures based on the immunization of the lambs. However, in order to succeed in the performance of the microbiological analyses it is important to consider several factors such as the sample to be taken, its conservation and the previous treatments of the sampled lamb.

In live lambs the samples can be taken by deep nasal swab or by bronchoalveolar lavage. These techniques allow obtaining information from live animals although they present limitations in the interpretation of the results. The main disadvantage of these samples is the poor correlation between the agents isolated from these samples and those obtained from samples taken at necropsy from the same animals. In addition, the proportion of cultures without growth is normally higher in bronchoalveolar lavages than in the samples taken during the necropsy (Valero et al., 2017).

The best results are obtained from samples taken at necropsy. According to our knowledge, results are optimized if the samples are taken considering the lesion pattern presented by the necropsied animal (Table 1). Whenever possible, it is preferable to analyse samples of consolidated lung lesions, while the injured regional lymph nodes will be the most indicated in the absence of a pneumonic lesion. The choice between delivery of injured organs or swabs depends on the mode of preservation that can be carried out until samples arrive to the laboratory (in case of difficulties, swabs are better) and interest in a specific point to be analysed (in this case, swabs are better).

4. Bacteria associated with ORC in lambs

Several infectious agents, including virus, parasites and bacteria have been associated with respiratory diseases in lambs. Some viruses such as parainfluenza 3 virus (Lehmkuhl and Cutlip, 1982; Sharp, 1990), respiratory syncytial virus (Wellemans, 1990) and border disease virus (Thabti et al., 2002) have also demonstrated their ability to reproduce the disease experimentally, however their role in natural outbreaks of ORC is poorly studied. On the contrary, the importance of bacteria in the development of the disease has been much better analysed. *Mannheimia haemolytica* (MH), *Pasteurella multocida* (PM), *Bibersteinia trehalosi* (BT) and *Mycoplasma* spp. are the most commonly isolated agents from bacterial pneumonias (Bell, 2008; Lacasta et al., 2008; Gonzalez et al., 2016). In Spanish fattening housed lambs that suffered ORC, MH, PM, BT, *Mycoplasma* spp. and *Escherichia coli* represented more than 80% of the isolates, however more than 80 different species were identified (Table 2).

148 However, these bacteria exist principally as commensal organisms of the nasopharynx and tonsils
149 of lambs. Furthermore, in microbiota studies, *Pasteurellaceae* and *Mycoplasma* have been found in
150 ovine healthy lungs (Glendinning et al., 2016). Nevertheless, it is under stressful conditions
151 (environmental, husbandry and management), immunodeficiency or respiratory viral-infection that they
152 can cause respiratory diseases (Brogden et al., 1998; Zecchinon et al., 2005). In relation to this point,
153 two studies were carried out by our group in order to confirm the presence of *Pasteurellaceae* by deep
154 swab of the nasal cavity of lambs without clinical signs. In the first one, 100 healthy lambs were sampled
155 on arrival at the feedlot and the presence of one or more species of *Pasteurellaceae* was identified in
156 50% of the lambs. In the second survey, 20 healthy lambs were analysed from the day of arrival at the
157 feedlot until their sale, taking samples every two weeks (4 samples per lamb). In this study it was found
158 that all the animals presented at least two positive samples throughout the fattening period, although
159 only three animals were positive during the whole period. These studies on feedlot lambs agree with the
160 results presented by the previous studies.
161 Another point that must be taken into account is that the growth of several species in the same
162 sample was the most common result in samples of fattening lambs. However, there were differences
163 among species; while in 26% of the BT isolates their culture was pure and massive, only 17%, 7% and
164 2% were pure cultures in MH, PM and *Mycoplasma* isolates, respectively (Gonzalez et al., 2016). In
165 any case, the cultures in which *Pasteurellaceae* and/or *Mycoplasmas* were identified represented 82%
166 out of the total of cultures with growth, for that reason, these agents are briefly described here below
167 (Table 3).
168
169 *4.1. Mannheimia haemolytica.*
170
171 *Mannheimia haemolytica* is the most frequent agent isolated from respiratory diseases of lambs
172 in different countries, production systems and ages. However, in a study carried out in Spain on 209
173 lung cultures of lambs of different ages, it was observed that the frequency of cultures in which MH was
174 identified was reduced from 59%, in lambs that died before 32 days of life, to 34%, in lambs that died
175 with more than 65 days of life. This reduction was even greater in the pure cultures of MH, where it was
176 reduced from 43% to 2% in the same study (González, 2016).
177 *Mannheimia haemolytica* is divided into 13 serotypes, among which, serotype A2 is the most
178 frequent in most of the studies performed on samples taken at necropsy (Frank, 1982; Prince et al., 1985;
179 Donachie, 1995; Vougidou et al., 2012; González et al., 2013). According to the experimental infection
180 studies conducted by Odugbo et al., serotypes A7, A9 and A2 were the most pathogenic (Odugbo et al.,
181 2004). This could explain the differences found on MH serotypes isolated in Spain according to the
182 origin of the samples. In the case of samples taken from animals died from ORC, the main serotype was
183 A2 (González et al., 2013), while serotype A7 was the most frequent when the sample was obtained

from slaughterhouse pneumonic lesions (Pinto, 2016) (Table 4). Moreover, A2 was the only serotype of MH isolated from hyperacute forms in Spain (González et al., 2013).

In addition, several of these serotypes can coexist in the same animal or flock. In this sense, a study published by Frank, showed, in 49 studied flocks, that in 65% of the cases more than one serotype was isolated from the same flock and in more than 8% of the flocks, at least 5 serotypes were detected (Frank, 1982). Moreover, in the study conducted by Pinto on samples obtained from pneumonic lesions at the slaughterhouse, it was observed that in 23% of the samples was identified more than one MH serotype (Pinto, 2016). This must be taken into account when establishing ORC control plans.

4.2. Bibersteinia trehalosi.

Formerly known as *Pasteurella haemolytica* T biotype, it is divided into four serotypes and it is located in the tonsils of healthy animals. BT is isolated mainly in lambs that die older than one month of life, since BT colonizes the tonsils from the first three weeks of life, replacing the MH population that was located in them (Al Sultan and Aitken, 1985). BT has generally been associated with septicemic pasteurellosis (Donachie, 2000), however, 15 serotype was isolated mainly from sheep with consolidated lung lesions while 4 serotype was found in sheep with septicaemia (Odendaal and Henton, 1995). The same results were reported for lambs in Spain (González et al., 2013).

4.3. Pasteurella multocida.

The frequency of isolation of PM varies according to the countries studied, while in a study carried out in the UK it represented 1% (Bell, 2008), in surveys carried out in Spain it signified 11% and 19% at necropsies of young lambs (Lacasta et al., 2008) or fattening lambs (Gonzalez et al., 2016), respectively. In addition, PM was the main isolated agent in consolidated lung lesions taken at abattoir in a study conducted in Mexico (Blanco-Viera et al., 1995). These data are corroborated by a study carried out on 209 cultures from necropsies of lambs, in which PM was identified in 11%, 28% and 67% of the samples of dead lambs younger than 28 days of age, between 28 and 65 days of life and older than 65 days, respectively (González, 2016).

Pasteurella multocida is divided into 5 serogroups, of which, serogroup B in Asia and serogroup E in Africa are associated with haemorrhagic septicaemia (Rajeev et al., 2011). Two studies carried out on lambs samples taken at the slaughterhouse found that serogroup A was the most frequently found, followed by serogroup D and no isolations of serogroups B or E were identified (Blanco-Viera et al., 1995; Pinto, 2016). This agrees with the data obtained from the necropsies of fattening lambs performed by González, in which PM was isolated mainly from consolidated lung lesions (Gonzalez, 2016).

220 *4.4. Mycoplasma* spp.

221

222 A large number of *Mycoplasmas* have been related to the presentation of lung lesions in sheep;
223 the most frequent are *Mycoplasma ovipneumoniae* and *Mycoplasma arginine* (Nicholas et al., 2008;
224 Chazel et al., 2010). However, defining the proportion of *Mycoplasmas* spp. found in the samples is
225 complicated due to the difficulties that its culture represents (Carmichael et al., 1972). Therefore, other
226 techniques have been proposed, such as immunohistochemical tests or the use of PCR tests (Kılıc et al.,
227 2013). The use of these techniques increases the detection of *Mycoplasma* spp. as it was shown in a
228 work carried out by Moreno, in which *Mycoplasma ovipneumoniae* was the agent most frequently
229 identified at slaughterhouse lung lesions when immunohistochemical techniques (Moreno, 1994) or
230 PCR (Lindström et al., 2018) were used. In other studies that used cultures to measure the frequency of
231 isolation, 28% of *Mycoplasma* spp.-positive samples were found when the origin was consolidated lung
232 lesions taken at the slaughterhouse (Luzón, 1999) or 14% when the samples were taken from
233 consolidated lung lesions during the necropsy (Gonzalez et al., 2016).

234 However, *Mycoplasmas* spp. are rarely isolated in pure culture, although consolidated lung lesions
235 were reproduced by experimental infection without the presence of other agents (Jones et al., 1982).
236 Recently, a study carried out at the University of Idaho under farm conditions investigate the role of
237 *Mycoplama ovipneumoniae*, and it was observed that the animals exposed to it presented a greater
238 number of clinical respiratory signs, increased microscopic pulmonary lesions in the slaughterhouse and
239 reduction of average daily growths during the fattening period (Besser et al., 2019). The mechanisms
240 that favour infection by other bacteria were defined by Niang et al., who observed that, in infected lambs,
241 the phagocytic capacity of the macrophages was reduced and the defence provided by the mucociliary
242 layer decreased (Niang et al., 1998). These data were corroborated by our team with a retrospective
243 study conducted on 402 samples taken from consolidated lung lesions of dead lambs due to ORC. It was
244 observed that the proportion of BT-positive cultures remained the same whether or not there was
245 presence of *Mycoplasma* spp. However, the proportion of MH identification increased from 60% to 69%
246 in the cultures in which *Mycoplasma* spp. was also found, although it was not statistically significant
247 (p=0.085). The differences were highly significant for the presence of PM. When *Mycoplasma* spp. was
248 also found in the culture, the risk to find PM in cultures increased 2.7 times (39% vs. 63%).

249

250 **5. Relationship between lesion pattern and aetiology in lambs with ORC.**

251

252 As a summary, it is possible to conclude that the aetiology presents differences among the clinical
253 presentations of ORC in lactating and fattening lambs (Table 5). First, hyperacute forms have a greater
254 variety of isolates than the rest of the forms and this is where the greatest number of pure cultures are
255 found. It should be noted that MH in pure culture was the agent most frequently isolated in this clinical
256 presentation during lactation, however during the fattening period pure culture of BT was the most

frequent isolate. In the acute forms, the MH culture was the most identified, in pure culture during lactation and in mixed culture with PM during the fattening period. In contrast, in chronic forms, PM was the most common agent in pure culture during lactation and in combination with MH in the fattening period. Furthermore, in lactation, MH was the most important agent in the hyperacute and acute forms and PM in chronic form, while in the fattening period the most frequent agents were BT, MH and PM for hyperacute, acute and chronic forms, respectively.

6. Concluding remarks.

There are several patterns of ORC lesion that correspond to different clinical pictures and that vary according to age of the lamb. The accurate identification of these patterns allows the veterinarian to guide a correct sampling and to know the bacteria most frequently isolated in this kind of lesions. However, these data should be valued as a guide that can never substitute the sampling for microbiological analysis, because all these agents can be present in all the clinical forms and mixed cultures are very recurrent.

Conflict of interest statement

The authors have nothing to disclose.

References

Al Sultan, I., Aitken, I.D., 1985. The tonsillar carriage of *Pasteurella haemolytica* in lambs. J. Comp. Pathol. 95, 193-201.

Bell, S., 2008. Respiratory disease in sheep - 1. Differential diagnosis and epidemiology. In Pract. 30, 200-207.

Benavides, J., González, L., Dagleish, M., Pérez, V., 2015. Diagnostic pathology in microbial diseases of sheep or goats. Vet. Microbiol. 181, 15-26.

Blanco-Viera, F.J., Trigo, F.J., Jaramillo-Meza, L., Aguilar-Romero, F., 1995. Serotypes of *Pasteurella multocida* and *Pasteurella haemolytica* isolated from pneumonic lesions in cattle and sheep from Mexico. Rev. Latinoam. Microbiol. 37, 121-126.

Brogden, K.A., Lehmkuhl, H.D., Cutlip, R.C., 1998. *Pasteurella haemolytica* complicated respiratory infections in sheep and goats. Vet. Res. 29, 233-254.

Besser T.E., Levy J., Ackerman M., Nelson D., Manlove K., Polter K.A., Busboom J., Benson M., 2019. A pilot study of the effects of *Mycoplasma ovipneumoniae* exposure on domestic lamb growth and performance. PLoS ONE 14(2): e0207420

Carmichael, L.E., St. George, T.D., Sullivan, N.D., Horsfall, N., 1972. Isolation, propagation, and characterization studies of an ovine *Mycoplasma* responsible for proliferative interstitial pneumonia. Cornell Vet. 62, 654-679.

Casteleyn, C., Breugelmans, S., Simoens, P., Broeck, V.d., 2011. The Tonsils revisited: Review of the anatomical localization and histological characteristics of the tonsils of domestic and laboratory animals. Clin. Dev. Immunol. 2011, Article ID 472460, 14 pages.

299 Chazel, M., Tardy, F., Le Grand, D., Calavas, D., Poumarat, F., 2010. Mycoplasmoses of ruminants in France:
300 recent data from the national surveillance network. BMC Vet. Res. 6:32.
301 De las Heras, M., Ferrer, L.M., 2001. Pasteurelosis ovina y caprina: control y prevención. In: Intervet., E.L. (Ed.),
302 Pasteurelosis ovina y caprina: control y prevención. Enfermedades por Clostridios en el ganado ovino.
303 Donachie, W., 1995. Vaccine development against *Pasteurella haemolytica* infections in sheep. In: Donachie, W.,
304 Lainson, F.A. and Hodgson, J.C. (Ed.), *Haemophilus*, *Actinobacillus* and *Pasteurella*, Plenum Publishing
305 Corporation, New York, pp. 25-37.
306 Donachie, W., 2000. Pasteurellosis. Diseases of sheep, Blackwell Science, Oxford, pp. 191-198.
307 Frank, G.H., 1982. Serotypes of *Pasteurella haemolytica* in sheep in the midwestern United States. Am. J. Vet.
308 Res. 43, 2035-2037.
309 Gilmour, N.J.L., Gilmour, J.S., 1985. Diagnosis of pasteurellosis in sheep. In Pract. 7, 145-146, 148-149.
310 Glendinning, L., Wright, S., Pollock, J., Tennant, P., Collie, D., McLachlan, G., 2016. Variability of the Sheep
311 Lung Microbiota. Appl. Environ. Microbiol. 82, 3225-3238.
312 González, J.M., 2016. Factores que condicionan la supervivencia de los corderos tipo ternasco. Estudio del
313 complejo respiratorio ovino: presentación, formas lesiones, agentes implicados y serotipificación de
314 *Pasteurella haemolytica*., Doctoral thesis. University of Zaragoza. Spain.
315 González, J.M., Bello, J.M., Rodríguez, M., Navarro, T., Lacasta, D., Fernández, A., De las Heras, M., 2016. Lamb
316 feedlot production in Spain: Most relevant health issues. Small Rumin. Res. 142, 83-87.
317 González, J.M., Lacasta, D., Ferrer, L.M., Figueras, L., Abadie, G., De las Heras, M., 2013. *Mannheimia*
318 *haemolytica* and *Bibersteinia trehalosi* serotypes isolated from lambs with ovine respiratory complex in
319 Spain. J. Hellenic Vet. Med. Soc. 64, 177-182.
320 Goodwin, K.A., Jackson, R., Brown, C., Davies, P.R., Morris, R.S., Perkins, N.R., 2004. Pneumonic lesions in
321 lambs in New Zealand: patterns of prevalence and effects on production. N Z Vet. J. 52, 175-179.
322 Hervas, J., Méndez, A., Gomez-Villamandos, J.C., Villalba, E., Diaz, E., Cano, T., Carrasco, L., Padro, J.M.,
323 Fernández, A., Sierra, M.A., 1996. Etiologic and pathologic study of respiratory disease in lambs from
324 intensive breeding facilities in southern Spain. J. Vet. Med. B Infect. Dis. Vet. Public Health 43, 221-231.
325 Jones, G.E., Field, A.C., Gilmour, J.S., Rae, A.G., Nettleton, P.F., McLauchlan, M., 1982. Effects of experimental
326 chronic pneumonia on bodyweight, feed intake and carcase composition of lambs. Vet. Rec. 110, 168-173.
327 Kılıc, A., Kalender, H., Eroksuz, H., Muz, A., Tasdemir, B., 2013. Identification by culture, PCR, and
328 immunohistochemistry of mycoplasmas and their molecular typing in sheep and lamb lungs with
329 pneumonia in Eastern Turkey. Trop. Anim. Health Prod. 45, 1525-1531.
330 Lacasta, D., Ferrer, L.M., Ramos, J.J., González, J.M., De las Heras, M., 2008. Influence of climatic factors on the
331 development of pneumonia in lambs. Small Rumin. Res. 80, 28-32.
332 Lindström, L., Tauni, F. A., Vargmar, K. 2018. Bronchopneumonia in Swedish lambs: a study of pathological
333 changes and bacteriological agents. Acta Vet. Scand. 60(1), 54.
334 Lehmkuhl, H.D., Cutlip, R.C., 1982. Characterization of parainfluenza type 3 virus isolated from the lung of a
335 lamb with pneumonia. Am. J. Vet. Res. 43, 626-628.
336 López, S., Martison, S.A., 2017. Respiratory System, mediastinum and pleura. In: Zachary, E.J. (Ed.), Pathologic
337 basis of Veterinary Disease., Elsevier, St. Louis.
338 Luzón, J., 1999. Influencia de las afecciones respiratorias en los principales parámetros productivos de los corderos
339 tipo ternasco. Doctoral thesis. University of Zaragoza. Spain.
340 McRae, K.M., Baird, H.J., Dodds, K.G., Bixley, M.J., Clarke, S.M., 2016. Incidence and heritability of ovine
341 pneumonia, and the relationship with production traits in New Zealand sheep. Small Rumin. Res. 145, 136-
342 141.
343 Moreno, B., 1994. Contribución al conocimiento de la neumonía del cordero. Doctoral thesis. University of
344 Zaragoza. Spain.
345 Navarro, T., Ramos, J.J., Figueras, L., González, J.M., 2018a. Epidemiology of ovine respiratory complex in
346 lambs. Small Rumin. Res. THIS ISSUE.
347 Niang, M., Rosenbusch, R.F., Andrews, J.J., Lopez-Virella, J., Kaeberle, M.L., 1998. Occurrence of autoantibodies
348 to cilia in lambs with a 'coughing syndrome'. Vet. Immunol. Immunopathol. 64, 191-205.
349 Nicholas, R.A.J., Ayling, R.D., Loria, G.R., 2008. Ovine mycoplasmal infections. Small Rumin. Res. 76, 92-98.
350 Odendaal, M.W., Henton, M.M., 1995. The distribution of *Pasteurella haemolytica* serotypes among cattle, sheep,
351 and goats in South Africa and their association with diseases. Onderstepoort J. Vet. Res. 62, 223-226.

Odugbo, M.O., Odama, L.E., Umoh, J.U., Lombin, L.H., 2004. The comparative pathogenicity of strains of eight serovars and untypable strains of *Mannheimia haemolytica* in experimental pneumonia of sheep. Vet. Res. 35, 661-669.

Pinto, C.E., 2016. Epidemiología molecular de las poblaciones bacterianas de "*Mannheimia haemolytica* y *Pasteurella multocida*" asociadas a la presencia de lesiones neumónicas en corderos en matadero. Docotral thesis. University Complutense of Madrid. Spain Prince, D.V., Clarke, J.K., Alley, M.R., 1985. Serotypes of *Pasteurella haemolytica* from the respiratory tract of sheep in New Zealand. N Z Vet. J. 33, 76-77.

Rajeev, R., Panda, S.K., Acharya, A.P., Singh, A.P., Gupta, M.K., 2011. Molecular diagnosis of haemorrhagic septicaemia - a review. Vet. World 4, 189-192.

Sharp, J.M., 1990. Parainluenza-3 virus in sheep. In: Dinter, Z., Morein, B. (Eds.), Virus Infections of Ruminants, Elsevier, Amsterdam pp. 335-339.

Thabti, F., Fronzaroli, L., Dlissi, E., Guibert, J.M., Hammami, S., Pepin, M., Russo, P., 2002. Experimental model of Border Disease Virus infection in lambs: comparative pathogenicity of pestiviruses isolated in France and Tunisia. Vet. Res. 33, 35-45.

Valero, M., Asín, J., Saura, F., Muñoz, A., Baselga, R., Ventura, J.A., Carreño, L., Ramo, M.A., 2017. Estudio comparativo de dos métodos de toma de muestras broncoalveolares para el diagnóstico de patologías respiratorias en ovino. In: Caprinotecnia., S.E.d.O.y. (Ed.), XLII Jornadas Científicas de la SEOC. SEOC, Salamanca, pp. 497-502.

Vougidou, C., Sandalakis, V., Psaroulaki, A., Petridou, E., Donachie, W., Ekateriniadou, L., 2012. Serotypic diversity and sequence variation of the ompA gene among *Mannheimia haemolytica* isolates from domestic ruminants. J. Hellenic Vet. Med. Soc. 63, 13-24.

Wellemans, G., 1990. Ovine and caprine respiratory syncytial virus. In: Dinter, Z., Morein, B. (Eds.), Virus Infections of Ruminants, Elsevier, Amsterdam pp. 377-378.

Zecchinon, L., Fett, T., Desmecht, D., 2005. How *Mannheimia haemolytica* defeats host defence through a kiss of death mechanism. Vet. Res. 36, 133-156.

Table 1. Practical recommendations for sampling of bacterial lesions associated with ORC in lambs during necropsy or slaughtering according to the pattern of lesions.

Lesion pattern	Lung	Regional lymph nodes	Brain*
Hyperacute (septicaemia)	--	Tissue / Swab	Swab
Acute or chronic (pneumonia)	Refrigerated / Frozen	Tissue / Swab	Swab
Abattoir (pneumonia)	Refrigerated	--	--

*If there is pus in meninges.

Table 2. Results of isolates from necropsies and abattoir samples related to ORC in lambs. 2,321 microbiological cultures were performed, with no bacterial growth in 422 samples (18.18%) and 3,827 isolates in total. Bacteria isolates have been aggregated into *Pseudomona, Moraxella, Streptococcus* and *Sthaphylococcus* genus for convenience.

Isolate	Frequency (%)
Mannheimia haemolytica	25%
Bibersteinia trehalosi	7%
Pasteurella multocida	21%
Mycoplasma spp.	16%
Escherichia coli	13%
Genus *Pseudomonas*	4%
Genus *Streptococcus*	3%
Genus *Moraxella*	2%
Genus *Staphylococcus*	2%
Others	7%

Table 3. Results of cultures from necropsies and abattoir samples related to ORC in lambs. 2,321 microbiology cultures were performed with 422 samples with no bacterial growth (1818%) and 3,827 isolates.

Culture	Presence of *Mycoplasma spp.*	Absence of *Mycoplasma spp.*
MH	2%	9%
PM	5%	3%
BT	1%	4%
My	1%	--
MH+PM	**13%**	**18%**
Mixed Paste	7%	**20%**
EC	--	4%
No Pasteurellas	--	13%

MH+PM: mixed culture of *Mannheimia haemolytica* and *Pasteurella multocida*; **MH**: pure culture of *Mannheimia haemolytica*; **MH+PM+My**: mixed culture of *Mannheimia haemolytica*, *Pasteurella multocida* and *Mycoplasma* spp.; **PM**: pure culture of *Pasteurella multocida*; **BT**: pure culture of *Bibersteinia trehalosi*; **My**: pure culture of *Mycoplasma* spp; **Mixed Paste**: mixed culture of *Pasteurellaceae* and other bacteria; **Mixed Paste + My**: mixed culture of *Pasteurellaceae, Mycoplasma* spp and other bacteria; **EC**: pure culture of *Escherichia coli*; **No Pasteurellas**: culture without *Pasteurellaceae* or *Mycoplasma* spp.

Table 4. Results of serotypes of *Mannheimia haemolytica* according to origin of samples: necropsy (86 isolates) or abattoir (92 isolates). It has been compiled from the studies of González et al., and Pinto, C.E. (González et al., 2013; Pinto, 2016).

Serotypes of *Mannheimia haemolytica*	Necropsy	Abattoir
A1	12%	3%
A2	**29%**	5%
A5	--	7%

A6	2%	11%
A7	2%	**13%**
A8	2%	5%
A9	4%	8%
A11	--	1%
A12	18%	8%
A13	--	1%
A14	2%	0%
A17	--	1%
NT*	29%	38%

*Not typable.

Table 5. Results of *Pasteurellaceae* cultures from necropsies samples related to ORC in lambs. 1,217 microbiology cultures were performed with 127 samples with no bacterial growth (10.43%). The results have been presented in percentages frequency according to the lesion pattern: hyperacute (123), acute (790) and chronic (177) and the moment of lamb death: lactation (217) vs. fattening period (873).

Culture	Hyperacute (22% no growth)		Acute (6% no growth)		Chronic (19% no growth)	
	Lactation	Fattening	Lactation	Fattening	Lactation	Fattening
MH + PM	--	13%	16%	**28%**	18%	**41%**
MH	**48%**	13%	**41%**	15%	29%	19%
PM	3%	--	22%	12%	**32%**	11%
BT	--	**46%**	--	9%	--	3%
MH + BT	--	--	--	7%	--	3%
Mixed Paste	25%	13%	3%	21%	9%	22%
No Pasteurellas	25%	17%	18%	7%	12%	3%

MH+PM: mixed culture of *Mannheimia haemolytica* and *Pasteurella multocida*; **MH**: pure culture of *Mannheimia haemolytica*; **PM**: pure culture of *Pasteurella multocida*; **BT**: pure culture of *Bibersteinia trehalosi*; **MH+BT**: mixed culture of *Mannheimia haemolytica* and *Bibersteinia trehalosi*; **Mixed Paste**: mixed culture of *Pasteurellaceae* and other bacteria; **No Pasteurellas**: culture without *Pasteurellaceae*.

Figure legends:

Figure 1. Most relevant gross lesions according to the ORC presentation form in lambs.