

Perinatal lamb mortality: Epidemiological and anatomopathological findings in Northeastern, Algeria

Mortalidad perinatal del cordero: Hallazgos epidemiológicos y anatomopatológicos en el noreste de Argelia

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ABSTRACT

Perinatal lamb affections are an essential entity that affects sheep flocks, especially when it has a fatal evolution. This study aims to identify the risk factors implied in perinatal lamb mortalities, describe the anatomopathological and histopathological characteristics of the viscera (lungs, livers, and kidneys) retrieved from twenty lamb corpses and the provided monitoring practices during the perinatal period. The statistical analysis demonstrated that the main factor implied in these mortalities was the hygiene of soil and livestock buildings ($P \leq 0.05$). Trachea inspection demonstrated the presence of foamy fluid ($n=9/20$) and amniotic fluid ($n=4/20$), grossly bronchopneumonia ($n=12/20$), interstitial pneumonia ($n=4/20$) and neonatal atelectasis ($n=4/20$). The livers showed acute hepatitis ($n=15/20$). Moreover, kidneys showed acute glomerulonephritis (membranoproliferative and glomerulosclerosis) ($n=13/20$), associated with hydronephrosis in six cases and epithelial nephritis in two cases ($n=2/20$) and hydronephrosis in two stillbirth cases ($n=2/20$). The principal histologic lesions observed on the lungs, livers, and kidneys were acute hyperemia, hemorrhage, inflammatory cell infiltration (neutrophils), and parenchyma destruction. In stillbirths, the presence of bright yellow materials (meconium fragments) in the lung parenchyma recognizes the involvement of meconium aspiration syndrome. The correlation between anatomopathological and histopathological results was essential in understanding the evolution of these affections and highlighting the conditions that led to the death. Furthermore, this study revealed a lack of preventive sanitary protocols, assisted lambing, and appropriate care strategies for lambs during their first days of life which contributed to the fatal evolution of these perinatal affections.

Key words: Bronchopneumonia; hepatitis; glomerulonephritis; meconium aspiration; perinatal mortalities

RESUMEN

Las afecciones perinatales en corderos son desórdenes patológicos que afecta a los rebaños ovinos, especialmente cuando presentan una gestación. Este estudio tiene como objetivo identificar los factores de riesgo implicados en las mortalidades perinatales de corderos, describir las características anatomopatológicas e histopatológicas de los órganos (pulmones, hígados y riñones) obtenidos en necropsia de veinte corderos y las prácticas de monitoreo proporcionadas durante el período perinatal. El análisis estadístico demostró que el principal factor implicado en estas mortalidades fue la higiene del suelo y de las instalaciones ganaderas ($P \leq 0,05$). La inspección de la tráquea demostró la presencia de líquido espumoso ($n=9/20$) y líquido amniótico ($n=4/20$), bronconeumonía ($n=12/20$), neumonía intersticial ($n=4/20$) y atelectasia neonatal ($n=4/20$). Los hígados mostraron hepatitis aguda ($n=15/20$), los riñones mostraron glomerulonefritis aguda (membranoproliferativa y glomerulosclerosis) ($n=13/20$), asociada con hidronefrosis en seis casos y nefritis epitelial en dos casos ($n=2/20$) e hidronefrosis en dos casos de muerte fetal ($n=2/20$). Las principales lesiones histológicas observadas en los pulmones, hígados y riñones fueron hiperemia aguda, hemorragia, infiltración de células inflamatorias (neutrófilos) y destrucción del parénquima. En los mortinatos, la presencia de materiales de color amarillo brillante (fragmentos de meconio) en el parénquima pulmonar reconoce la participación del síndrome de aspiración de meconio. La correlación entre los resultados anatomopatológicos e histopatológicos fue esencial para comprender la evolución de estas patologías perinatales, y su efecto sobre las condiciones que llevaron a la muerte. Además, este estudio reveló una ausencia de partos asistidos y estrategias de cuidado adecuadas a los corderos durante sus primeros días de vida lo que contribuyó a la evolución fatal de estas patologías perinatales.

Palabras clave: Bronconeumonía; hepatitis; glomerulonefritis; aspiración de meconio; mortalidades perinatales.

INTRODUCTION

Sheep flock productivity is an essential factor that evaluates the economic profitability of farms, especially in sheep-rearing (*Ovis aries*) countries (China, India, Iran, Nigeria, Ethiopia, and Algeria) [1, 2]. This productivity could be impacted by several affections that may have an infectious or non-infectious origin and touch many tracts (gastrointestinal, respiratory, or urinary) causing lamb mortality and decreased growth rate and production [3]. Furthermore, it was reported that these mortality causes have various distributions worldwide, but they remain similar (trauma, stillbirths, and infectious disease) [4, 5, 6], and mainly associated with several risk factors that are related to the environment (hygiene), the ewe (age, body score condition), or the lamb (age, sex), in addition to the breeding management system [7].

Regardless of their etiology, infections like pneumonia, omphalitis, and septicemia are the principal causes of mortality and low productivity in sheep farms [5, 8]. Depending on the infection severity, the immune response of the animal, and the management of cases, evolutionary pathways can go from recovery (with or without the restitution of the organ function) to a complete loss of the function that can be fatal for the animal [9, 10].

In Algeria, data on lamb's early life affections and stillbirths are limited. Therefore, due to its sheep-producing status, the conduction of a study on fatal perinatal lambs' affections targeting the dead lambs' population (a few hours to thirty days of age) is very relevant. This work aims to identify the risk factors associated with these mortalities, point out the provided monitoring practices during the perinatal period, describe the evaluation of macroscopic and microscopic lesions of the viscera of dead lambs, highlight the principal changes occurring during these affections and stillbirths, and understand their evolution to encourage further investigations on lamb's perinatal affections.

MATERIALS AND METHODS

Ethical approval

The scientific council of the Institute of Veterinary Sciences (Mentouri Brothers University, Constantine 1, Algeria) approved this study on the population of dead lambs.

Study area and sample collecting

The present study took place in Constantine (Northeastern Algeria) from December 2021 to December 2022. The survey included thirteen farms chosen for easy access and the cooperation of the owners. The collected corpses of lambs (a few hours to thirty days of age) were transported on ice packs, without further delay, to the necropsy room of the veterinary institute to perform a post-mortem examination. The number of retrieved corpses was governed by the capacity of the laboratory to process samples.

Epidemiological study

This survey involves a descriptive cross-sectional study focusing on thirteen sheep farms, covering all the localities

and the majority of farms. The choice of farms was guided by the free manager's acceptance to cooperate or not with the study. The epidemiological questionnaire of farms contained 76 closed-type questions. These were related to the infrastructure, food, hygiene, care, and supervision of the lambs on each farm. The information thus gathered was based both on personal observations and on data collected from the owners.

Anatomopathological and histopathological study

Necropsy

To carry out an anatomopathological and histopathological investigation. A post-mortem examination was performed on the selected lamb bodies (good preservation status), involving a visual inspection of viscera (lungs, trachea, liver, and kidney), palpation, and systematic incisions to describe the observed lesions [5, 11, 12], followed by the conservation of samples (n=112).

Slide preparation for histopathological study

Samples were fixed in formaldehyde (MZK BactChim, Algeria). After 24h-48h, they were carried out the standard hematoxylin-eosin staining (Sigma-Aldrich, USA) according to the following protocol: the fixed specimens were trimmed and transferred to labeled cassettes, then dehydrated (Thermo Scientific Citadel 2000, Model: 69800004, UK) in ethanol baths (MZK BactChim, Algeria) of increasing concentration, cleared with xylene and embedded (Thermo Scientific Histostar, Model: A81000001, UK) in paraffin (Oxford, USA). After that, the microtome (Leica Manual rotatory microtome, Model: RM2235, Germany) was used to section tissue slices approximately 4-5 micrometers thick that were deparaffinized with xylene (MZK BactChim, Algeria), rehydrated (Thermo Scientific Citadel 2000, Model: 69800004, UK) in ethanol baths (MZK BactChim, Algeria) of decreasing concentration and transferred to glass slides to be stained with hematoxylin and eosin and assembled to allow microscopic observation (Leica Phase Contrast and Darkfield trinocular Microscope with camera, Model: DM1000, Germany) [13].

Statistical analysis

All the collected data from the survey was incorporated into a contingency table to estimate the dependence between perinatal mortality record and the potential risk factors: hygiene of the soil and the livestock buildings, reproduction system, use of allotment, among others.

Risk factors associated with perinatal lamb mortality were assessed by the Fisher exact test (at 95% CI and $P < 0.05$). All statistical analyses were performed using the R studio software (7d165dcf, 2022-12-03 Build 353).

RESULTS AND DISCUSSION

Epidemiological study

Characteristics of the farms studied

The thirteen farms have Ouled Djellal breed, seven belong to the public sector and six to the private sector, their rearing capacity varies from 806 to 66 sheep per farm, most of them have two mating seasons per year ($n=8/13$) and for the remaining farms ($n=5/13$) rams are continuously rearing with the ewes (one to two mating seasons).

In the majority of farms, the breeding system was semi-extensive to extensive. To prepare the animals for the breeding season, some farms performed the flushing (feeding of extra concentrate to ewes 3 or 4 weeks before breeding.) and Crutching (Removing the wool around the perineal region and base of the tail of ewes to facilitate the mating). For mating, the used methods were pen mating for eight farms with an average ram-to-ewe ratio of 1:28 (one ram for 28 ewes) and flock mating for five farms.

The reproductive management especially during the perinatal period, showed a lack of monitoring and assistance to the ewes and the newborn lambs, which predisposes the ewes to experience a difficult or prolonged delivery causing various injuries and hypoxia to the lamb that can either die during the lambing (stillborn) or survive with poorer neonatal behavior (abilities to stand, suck, and to maintain body temperature) and delay or compromise maternal behavior expression (licking and grooming their offspring) [14].

The statistical analysis showed that the principal risk factor associated to the occurrence of lamb mortalities is the hygiene of soil and livestock buildings ($P \leq 0.05$) (TABLE I).

TABLE I Risk factors determining lamb mortality record.	
Risk factors	Determining mortality record P-value
Hygiene of the soil and the livestock buildings	0.004662
Reproduction system	0.07537
Allotment of ewes according to their stage (Empty / Full)	0.07537
Allotment of lambs according to their age	0.07537
Amount of feed available for the ewes	0.2929
Management of the lambs (cleaning of the umbilical cord)	1
Month of birth	0.5866

Which means that the hygiene of the soil and the livestock buildings constitutes a significant factor exposing the newborn lambs to pathogens that can be responsible for different affections (pneumonia, omphalitis, septicemia or diarrhea) [7]. Moreover, in many studies, sanitary practices were associated with mortality records. In France, more than 40% of the farmers do not disinfect livestock buildings [15]. In the UK, poor mothering-pen hygiene was correlated with a high perinatal mortality rate [16]. Thus, in Sudan, a study showed that hygiene was not a significant risk factor associated with lamb mortality [17].

On the other hand, it was noted that the mortalities were recorded during the Autumn-winter season, including lambs of various ages (few hours –30 d). (FIG. 1).

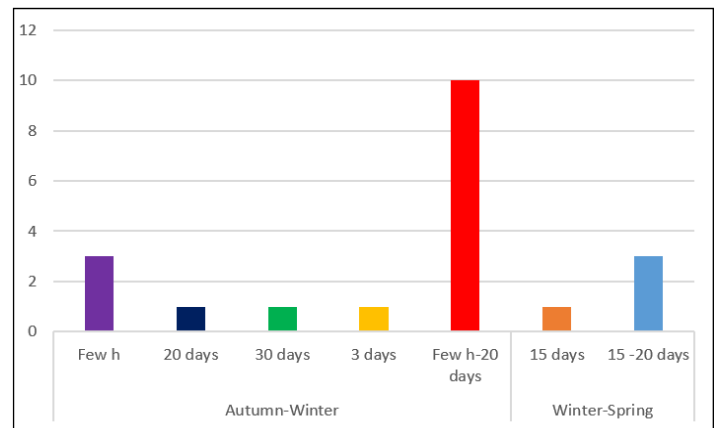


FIGURE 1. Lamb deaths according to lambing season (the number of lambs dying during autumn-winter season (few h- 30 d) is higher than winter-spring (15 d- 20 d))

Most mortalities were reported during the Autumn-winter season correlating with the study of Ibrahim *et al.*, [17]. Also in Nigeria, high mortality rates were observed during the wet season, which is considerate as common due to the inclement weather that can expose newborn lambs to hypothermia and respiratory tract affections [18]. Furthermore, the mortality rate worldwide was estimated to be ranged from 9 to 20%, with more than 50% of those deaths occurring in the first days after birth [16,19]. Moreover, Studies in Kenya by Wilson *et al.*, [20] indicated that up to 20% of lambs are lost by the first month of life which means that this period constitutes the most critical period in a newborn life because the infectious risk is the highest, and the immune response lacks efficiency [18, 21].

Anatomopathological findings

Twenty lamb bodies ($n=20/27$) were autopsied (good preservation status). The twenty respiratory tracts showed various lesions touching the trachea, lungs, and pleura.

Trachea inspection revealed the presence of foamy fluid (45%) ($n=9/20$) and amniotic fluid (20%) ($n=4/20$). Thus, the lungs showed pneumonia (80%) ($n=16/20$) and neonatal atelectasis (20%) ($n=4/20$).

The bronchopneumonia was the most commonly observed type of pneumonia ($n=12/16$). Gross lesions were congestion, hemorrhage (focal or extensive), hypertrophy, consolidation (red and grey hepatization), either focal or widespread, and edema extended to the whole lungs, the right, the left one, or eventually in some lobes.

Lung parenchyma incision ($n=4/12$) showed the presence of multiple abscesses containing whitish-yellow and greenish-colored pus, in addition to fibrinous pleuritis ($n=6/12$) located in the caudal, cranial, and middle lobes.

Interstitial pneumonia in the remaining four respiratory tracts ($n=4/16$) showed extensive congestion, hemorrhage with

firm and elastic consistency, a dark red color, and prominent rib impressions.

Neonatal atelectasis (n=4/20) showed incomplete distension of alveoli (lungs). Furthermore, the inspection of livers showed that acute hepatitis was observed in fifteen cases (n=15/20), the macroscopic lesions were congestion, dark or pale coloration, hemorrhage, hypertrophy, the presence of abscesses, and fibrinous deposit in only one case. Moreover, kidneys showed acute glomerulonephritis (n=13/20), grossly hypertrophy, congestion, petechiae, and cysts were observed on the cortex, and pale color, atrophy or hypertrophy of the medulla with sometimes a muddy consistency, associated with hydronephrosis in six cases. In the remaining two cases (n=2/20), there was epithelial nephritis (pale cortex and congestion of the medulla), and in two cases of stillbirths (n=2/20) hydronephrosis.

Histopathological findings

One hundred and twelve histologic sections of the lungs, livers, and kidneys were obtained and meticulously examined. All of the observed sections confirmed that it's an acute inflammatory reaction characterized by active hyperemia, and extensive hemorrhage in all the organs, as follows:

In the lungs, there was acute bronchiolitis (presence of inflammatory cells and exudate in the lumen), bronchiolar necrosis (destruction of the epithelium), edema (infiltration of the lung parenchyma with inflammatory cells such as neutrophils, macrophages, and red blood cells), alveolitis (alveoli filled with inflammatory exudate, and neutrophils), consolidation (replacement of the fluid exudate by a fibrinocellular one composed of fibrin, neutrophils, macrophages, and debris), and the loss of the alveolar structure of the lung (extensive fibrosis). Moreover, extensive necrosis complicated nine cases. Interstitial pneumonia slides showed active hyperemia, infiltration of the interstitium with inflammatory cells, cellular debris, and focal necrosis.

Neonatal atelectasis tissues showed acute hyperemia, infiltration of the lung parenchyma with inflammatory cells, and incomplete alveoli distention. In addition to a bright yellow material (pieces of meconium) and extensive necrosis in one case.

Livers observation revealed acute hepatitis (the presence of inflammatory cells especially neutrophils, red blood cells, and exudate in hepatic sinusoids and portal area, hepatocyte necrosis, and destruction of the parenchyma. In the abscess areas inflammatory cells, cell debris, and clusters of microorganisms are visible).

The kidneys section slides showed the presence of exudate, and a large area of necrosis (destruction of glomeruli, and urinary ducts). In addition, the thirteen cases of glomerulonephritis are of two types membranoproliferative (n=3/13) and glomerulosclerosis (n=10/13) besides two cases of epithelial nephritis. Some of the histopathological findings are illustrated in FIG. 2.

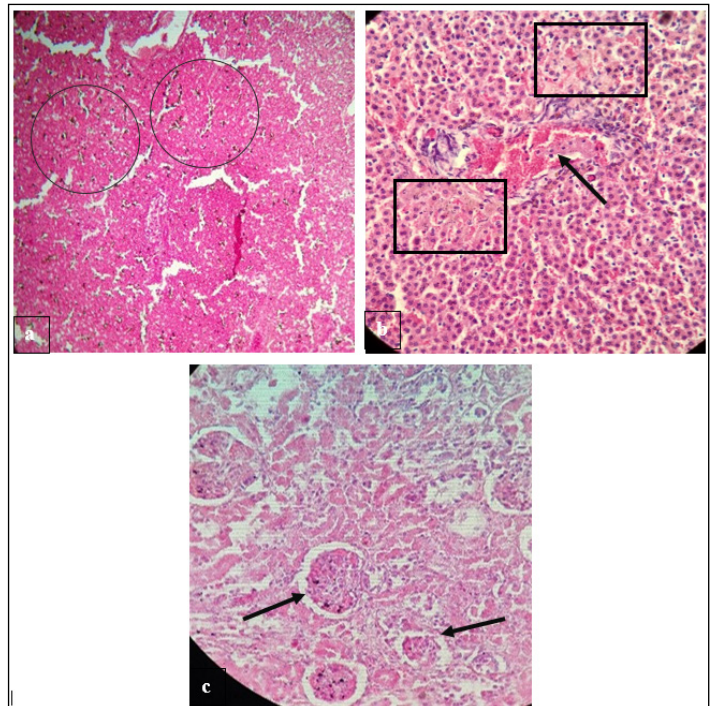


FIGURE 2. Observed lesions. (a) Neonatal atelectasis with bright yellow material (meconium pieces) (circles) $\times 80$. (b) Acute hepatitis with active congestion and inflammatory cells (arrow) and the destruction of the parenchyma (rectangles) $\times 200$. (c) Glomerulosclerosis with the shrinkage and hyalinization and complete loss of glomerular structures (arrows) $\times 200$

This study concluded that in 80% of the lungs (n=16/20), bronchopneumonia (n=12/16) is the most prominent affection with an evolution from a suppurative form to a fibrinous one, followed by interstitial pneumonia (n=4/16), matching other studies conducted on small ruminants lungs, in Sweden [22], Nigeria [23], Iran [3], and Turkey [24]. Therefore, both anatomopathological and histopathological findings showed that in the lungs, most bronchopneumonia cases are suppurative, and some have evolved into fibrinous bronchopneumonia (fibrinous pleuritis). These cases of pneumonia showed various stages of evolution: congestion, red hepatization, grey hepatization, and resolution represented by fibrosis, and in some cases complicated by extensive necrosis, interstitial pneumonia cases were acute, showing a more homogenous evolution of the inflammation in early stages (congestion and inflammatory cell infiltration) with necrosis foci [25, 26, 27], corresponding to the gross and histological findings of various studies conducted in Egypt [28], Nigeria [23], Iran [3], and Turkey [24] on lambs, sheep (*Ovis aries*), and goats (*Capra hircus*). The evolution of respiratory tract affections depends on the severity of the infection, which can cause reversible or irreversible injuries, the immune status of the animal, and the care and treatments provided. Case management is crucial, especially in lambs, since they have passive immunity, and their immune system develops during their first days of life (colostrum intake). Due to the insufficient effectiveness of the immune response, sepsis or death can occur quickly and lead to considerable losses in sheep flocks [26, 28].

However, in neonatal atelectasis cases (n=4/20), gross features show that the lamb aspirated amniotic fluid, indicating cases of stillbirths. Therefore, histopathological findings pointed out atelectasis and the presence of bright yellow material, which is meconium pieces, exhibiting meconium aspiration syndrome. This syndrome constitutes the most common cause of neonatal respiratory distress, primarily caused by hypoxia (during dystocia or prolonged parturition) that generates the relaxation of the anal sphincter and the release of meconium in the amniotic fluid. The lamb, gasping for air, aspirates this contaminated fluid [26, 29]. It's worth noting that this syndrome is a common problem in both human and animal neonates.

In humans, the incidence varies from low rates in developed countries to higher rates in developing ones. However, morbidity and mortality have declined thanks to new therapies and appropriate management of these cases [30, 31]. Even though cases were reported in piglets, puppies, calves, and foals, meconium aspiration syndrome received minor attention in the veterinary field [26]. This syndrome can cause airway obstruction, atelectasis, chemical pneumonitis, hypoxemia, acidosis, and pulmonary hypertension that can lead to death or the survival of the animal with nervous sequelae or hyperreactivity in the airways [30], which makes the conduct of studies on this entity very relevant.

Livers, presented acute hepatitis with neutrophils foci, exudate, and large areas of necrosis. It was noted that neutrophil accumulation is a response to chemotactic stimuli during bacterial and protozoal infections. In neonates (calves, lambs, and foals), some bacteria, such as *Escherichia coli* seed the liver via the umbilical veins or portal venous or hepatic arterial systems, and colonize the organisms via blood, causing sepsis that can be fatal to the newborn lamb [26].

Kidneys showed two types of glomerulonephritis membranoproliferative (hypercellularity due to the increase of inflammatory cells and generalized hyaline thickening

of the glomerular capillary basement membranes) and glomerulosclerosis (hypocellularity, shrinkage and hyalinization and complete loss of glomerular structures) as described in a study on ovine kidney lesions in Tikrit City; these lesions can occur during an infectious process or after a variable latent period causing kidney failure [32, 33] or have a spontaneous development as reported in Finn cross lambs from Alberta [34]. Besides epithelial nephritis (destruction of glomeruli, and urinary ducts), are mostly found in cases of nephrotoxicity due to an overuse of some antibacterial and antifungal agents such as Gentamicin, Neomycin, Streptomycin, and Tetracycline [35].

Lesion assessment of the touched organs revealed that in the majority of cases (n=15/20) lungs, liver and kidneys showed acute lesions, in the remaining cases lungs and kidney (n=3/20) and lungs only in two cases (TABLE II), which demonstrates the predomination of septicemic evolution.

Moreover, the anatomopathological and histopathological features showed that the majority of dead lambs had various lesions on the lungs, livers, and kidneys suggesting an infectious implication in these mortalities with a septicemic evolution that can rapidly occur during the first days of life [26, 28].

CONCLUSION

The conducted epidemiological and anatomopathological study established that the hygiene of soil and livestock buildings is the leading risk factor in perinatal lamb mortality registration in Constantine, Algeria, on the autumn-winter season, emphasized by the lack of preventive sanitary protocols (cleaning the mothering and lambing pens, changing their litter, and cleaning every material that may come into contact with the newborns). As well as, assisted lambing. That enhanced the risk of exposure to potential pathogens and the threaten of facing a difficult or prolonged delivery leading to various infections, stillbirths and compromising the expression of mother and neonatal behavior.

TABLE II
Lesion assessment of the touched organs per lamb corpses

Touched organs	Lesion assessment	Number of lamb corpses
Lungs, Liver and Kidneys	Bronchopneumonia +Acute hepatitis+ Glomerulonephritis (glomerulosclerosis)	5
	Bronchopneumonia +Acute hepatitis+ Glomerulonephritis (membranoproliferative)	2
	Bronchopneumonia +Acute hepatitis+ Glomerulonephritis (glomerulosclerosis) +Congenital hydronephrosis	2
	Bronchopneumonia +Acute hepatitis+ Epithelial nephritis	1
	Bronchopneumonia +Acute hepatitis+ Congenital hydronephrosis	1
	Interstitial pneumonia +Acute hepatitis+ Glomerulonephritis (glomerulosclerosis) +Congenital hydronephrosis	2
	Interstitial pneumonia +Acute hepatitis+ Glomerulonephritis (glomerulosclerosis)	1
	Interstitial pneumonia +Acute hepatitis+ Glomerulonephritis (membranoproliferative) + Congenital hydronephrosis	1
Lungs and Kidneys	Bronchopneumonia+ Epithelial nephritis	1
	Fetal atelectasis+ Congenital hydronephrosis	2
Lungs	Fetal atelectasis	2
Total		20

Furthermore, in most cases, lungs, livers, and kidneys showed multiple lesions exhibiting acute and severe evolution of pneumonia and septicemia that were fatal to these lambs. Moreover, this study demonstrated an implication of meconium aspiration syndrome in stillborns based on histopathological features (bright yellow materials). Highlighting that monitoring lambs during perinatal period is critical.

Further microbiologic investigations are needed to identify the pathogens implied in these mortalities and provide adequate and efficient treatment for these perinatal affections associated with appropriate monitoring practices during the perinatal period to avoid important lamb losses that can affect the sheep flock productivity.

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Authors' Contributions

H.B has actively worked on the necropsy of lambs, data interpretation, and drafting the paper; S.D designed, performed the experiments, and revised the paper; H.B and A.T contributed to the slide preparation for histopathological study, and L.B performed histologic evaluations.

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