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που εκπονήθηκε στην Κλινική Μαιευτικής και Αναπαραγωγής
του Τμήματος Κτηνιατρικής του Πανεπιστημίου Θεσσαλίας

Καρδίτσα 2022

Αφιερώνεται στην οικογένειά μου.

ΠΕΡΙΛΗΨΗ

Οι ειδικότεροι στόχοι της παρούσας διατριβής ήταν οι εξής: (α) η διερεύνηση της θνησιμότητας σε θηλυκά πρόβατα κατά την περί τον τοκετό περίοδο, σε μία έρευνα πεδίου σε εκτροφές προβάτων, (β) η διαπίστωση της ειδικότερης χρονικής περιόδου σε σχέση με τον τοκετό, οπότε συνέβαιναν αυτά τα περιστατικά θανάτου, (γ) η ταυτοποίηση των παθολογικών καταστάσεων που σχετίζονταν με τα περιστατικά θανάτου και (δ) η εκτίμηση παραγόντων επικινδυνότητας για αυτά τα περιστατικά θανάτου. Η διατριβή χωρίζεται σε δύο κεφάλαια και ακολουθεί η γενική συζήτηση.

Στο πρώτο κεφάλαιο, ανασκοπείται η σχετική, περιορισμένη βιβλιογραφία αναφορικά με περιστατικά θανάτου θηλυκών προβάτων κατά την περί τον τοκετό περίοδο.

Στο δεύτερο κεφάλαιο, περιγράφεται μία μελέτη πεδίου σε περιστατικά θανάτου θηλυκών προβάτων κατά την περί τον τοκετό περίοδο. Η μελέτη πραγματοποιήθηκε σε 60 προβατοτροφικές εκμεταλλεύσεις για χρονικό διάστημα δύο ετών.

Κατά την έναρξη καθενός από τα δύο έτη της μελέτης πεδίου υπήρχαν συνολικά 13.418 και 12.599 θηλυκά πρόβατα, αντίστοιχα, στις 60 εκτροφές. Σε κάθε ζώο ατομικά, η περί τον τοκετό περίοδος καθορίστηκε από 10 ημέρες πριν από τον τοκετό έως 7 μέρες μετά από αυτόν (δηλαδή, συνολική διάρκεια 18 ημέρες). Υπολογίστηκαν μέτρα συχνότητας εμφάνισης περιστατικών θανάτου κατά την περί τον τοκετό περίοδο, καθώς και κατά καθεμία από τις τρεις χρονικές υποπεριόδους αυτής (πριν, κατά, μετά τον τοκετό). Σε όλα τα περιστατικά, πραγματοποιήθηκαν οι κατάλληλες παρακλινικές εξετάσεις (π.χ., νεκροτομική εξέταση, μικροβιολογική εξέταση ιστοτεμαχίων) και καθορίστηκε ο χρόνος θανάτου κάθε ζώου σε σχέση με τον τοκετό (δηλαδή: πριν, κατά ή μετά από αυτόν). Με βάση τα ευρήματα των κατά περίπτωση περαιτέρω εξετάσεων, έγινε διάγνωση της παθολογικής κατάστασης που σχετιζόταν με το περιστατικό. Στη συνέχεια, συνολικά 15 παράγοντες (που αφορούσαν στην εκτροφή) αξιολογήθηκαν για δυνητική συσχέτιση με τα περιστατικά θανάτου κατά την περί τον τοκετό περίοδο. Πραγματοποιήθηκαν μονοπαραγοντικές και πολυπαραγοντικές αναλύσεις για την αξιολόγηση παραγόντων επικινδυνότητας που σχετίζονταν με τα περιστατικά θανάτου κατά την περί τον τοκετό περίοδο.

Σε όλη τη διάρκεια της μελέτης (δύο έτη), στις 60 εκτροφές καταγράφηκαν συνολικά 345 περιστατικά θανάτου σε ενήλικα θηλυκά πρόβατα. Η μέση συχνότητα περιστατικών θανάτου στη διάρκεια αυτών των δύο ετών ήταν 1,3%. Από αυτά, 147 περιστατικά συνέβησαν εκτός της περί τον τοκετό περιόδου και 198 περιστατικά (57,4% του συνόλου των περιστατικών θανάτου) συνέβησαν στη διάρκεια της περί τον τοκετό περιόδου ($P < 0.0001$ συγκριτικά με τα περιστατικά θανάτου εκτός

της εν λόγω περιόδου). Η μέση συχνότητα περιστατικών θανάτου κατά την περί τον τοκετό περίοδο σε όλη τη διάρκεια των δύο ετών ήταν 0,7%. Τα περισσότερα περιστατικά θανάτου (n = 135, 68,2%) συνέβησαν πριν από τον τοκετό, ενώ συνέβησαν λιγότερα κατά (n = 11, 5,6%) ή μετά (n = 52, 26,3%) από αυτόν. Η ανά ημέρα συχνότητα περιστατικών θανάτου, σε καθεμία από τις τρεις χρονικές υποπεριόδους της όλης περί τον τοκετό περιόδου, ήταν 0,05% πριν από τον τοκετό, 0,04% κατά τον τοκετό και 0,03% μετά τον τοκετό (P = 0.001). Η τοξιναιμία εγκυμοσύνης ήταν το κλινικό πρόβλημα που σχετιζόταν συχνότερα με περιστατικά θανάτου κατά την περί τον τοκετό περίοδο (41,4% όλων των περιστατικών θανάτου, 60,7% των περιστατικών θανάτου πριν από τον τοκετό). Άλλες παθολογικές καταστάσεις που σχετίστηκαν με περιστατικά θανάτου ήταν η δυσπεπτική οξέωση (11,6% όλων των περιστατικών θανάτου, 17,0% των περιστατικών θανάτου πριν από τον τοκετό), οι παθολογικές καταστάσεις της γεννητικής οδού (11,6% όλων των περιστατικών θανάτου, 44,2% των περιστατικών θανάτου μετά τον τοκετό), η οξεία κλινική μαστίτιδα (10,6% όλων των περιστατικών θανάτου, 40,4% των περιστατικών θανάτου μετά τον τοκετό), οι λοιμώξεις του αναπνευστικού συστήματος (9,6% όλων των περιστατικών θανάτου, 14,1% των περιστατικών θανάτου πριν από τον τοκετό) και η δυστοκία (5,6% όλων των περιστατικών θανάτου, 100,0% των περιστατικών θανάτου μετά τον τοκετό). Από τα 11 ζώα που πέθαναν κατά τον τοκετό, τα 6 (54,5%) βρίσκονταν στον πρώτο τοκετό τους. Η αναλογία τους ήταν σημαντικά μεγαλύτερη από ανάλογα ζώα που πέθαναν πριν από ή μετά τον τοκετό (8,0%) (P < 0.0001). Κατά την πολυπαραγοντική ανάλυση, βρέθηκε ότι ο αυξημένος δείκτης πολυδυμίας σε κάποια εκτροφή (μέση τιμή > 1,50 αρνιά ανά προβατίνα) ήταν ο μόνος παράγοντας επικινδυνότητας για περιστατικά θανάτου θηλυκών προβάτων κατά την περί τον τοκετό περίοδο (P = 0.035).

Τα συμπεράσματα που προκύπτουν από τα ευρήματα αυτής της διατριβής, είναι τα παρακάτω.

(α) Περιστατικά θανάτου κατά την περί τον τοκετό περίοδο συμβαίνουν σποραδικά σε εκτροφές προβάτων, τα περισσότερα δε από αυτά πριν από τον τοκετό.

(β) Η τοξιναιμία εγκυμοσύνης είναι το κλινικό πρόβλημα που σχετίζεται συχνότερα με περιστατικά θανάτου κατά τη συγκεκριμένη περίοδο.

(γ) Ο κύριος παράγοντας επικινδυνότητας για θνησιμότητα κατά την περί τον τοκετό περίοδο είναι ο αυξημένος δείκτης πολυδυμίας σε κάποια εκτροφή (> 1,50 αρνιά ανά προβατίνα).

Με βάση την κείμενη νομοθεσία και μετά από σχετική απόφαση στη με αριθμό 103/04.08.2021 συνεδρίαση της Συνέλευσης του Τμήματος Κτηνιατρικής του Πανεπιστημίου Θεσσαλίας, η συγγραφή της διατριβής έγινε στην αγγλική γλώσσα.

Δημοσιεύσεις σχετιζόμενες με την παρούσα διατριβή

Στην παρακάτω επιστημονική δημοσίευση παρουσιάζεται τμήματα της παρούσας διατριβής: **I.** A.P. Politis, N.G.C. Vasieliou, P.J. Cripps, D.V. Liagka, P.T. Boufis, I. Valasi, V.S. Mavrogianni, G.C. Fthenakis (2021) "Mortality of dairy sheep during the peri-parturient period: results of a field investigation in Greece" *Animals*, 11:2172.

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Γ.Χ. Φθενάκης, Καθηγητής	Τμήμα Κτηνιατρικής, Πανεπιστήμιο Θεσσαλίας
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Π.Γ. Γκουλέτσου, Αναπληρώτρια καθηγήτρια	Τμήμα Κτηνιατρικής, Πανεπιστήμιο Θεσσαλίας
Β.Σ. Μαυρογιάννη, Αναπληρώτρια καθηγήτρια	Τμήμα Κτηνιατρικής, Πανεπιστήμιο Θεσσαλίας
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UNIVERSITY OF THESSALY

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FACULTY OF VETERINARY MEDICINE

DISEASES AND MORTALITY OF EWES DURING THE PERI-PARTURIENT PERIOD: FREQUENCY MEASURES AND RISK FACTORS

ANTONIS P. POLITIS

DVM (Thessaloniki)

A THESIS SUBMITTED FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

Work carried out at the Department of Obstetrics and Reproduction
of the Faculty of Veterinary Medicine of the University of Thessaly

Karditsa, Greece 2022

ABSTRACT

The specific objectives of this thesis are as follows: (a) the investigation of the incidence risk of peri-parturient mortality in a field investigation in flocks in Greece, (b) the study of the time that such mortality occurs in relation to lambing, (c) the identification of the pathological conditions more frequently associated with the deaths and (d) the evaluation of factors potentially associated with death during the peri-parturient period. The thesis is divided in two chapters, followed by the general discussion.

In the first chapter, the limited relevant literature regarding death of ewes during the peri-parturient period is reviewed.

In the second chapter, a field study into the mortality of ewes during the peri-parturient period is described. The study involved 60 flocks for a period of two years.

In total, there were 13,418 and 13,599 ewes at the start of each of the two study years, respectively, in the 60 flocks. At the individual animal level, the peri-parturient period was defined from 10 days before to 7 days after lambing (i.e., a total duration of 18 days). In all the ewes that died during that period, a detailed post-mortem examination was performed. The time of death in relation to lambing was established. Following the appropriate paraclinical tests on relevant samples (e.g., post-mortem examination, microbiological examination of tissue samples) and based on their results, a diagnosis was made of the pathological condition in the ewe. In total, 15 variables (flock-level factors) were evaluated for potential association with deaths during the peri-parturient period. In relation to the time of occurrence within each year of the study, death was classified as having occurred before, during or after lambing. The incidence rates of death during the peri-parturient period during the entire peri-parturient period, as well as in each of the three components of the period (before, at, after lambing) were established. Univariable and multivariable analyses were performed to detect risks factors associated with death during the peri-parturient period.

A total of 345 deaths of adult ewes were recorded during the two years of the study; the average incidence risk during the two years was 1.3%. There were 147 deaths outside the peri-parturient period, whilst a total of 198 deaths (57.4% of all deaths) occurred during the peri-parturient period ($P < 0.0001$ compared to deaths that occurred outside the peri-parturient period). The average incidence risk of death during the peri-parturient period during the two years was 0.7%. Most cases of death (n = 135; 68.2%) occurred before lambing and fewer ones at (n = 11; 5.6%) or after (n = 52; 26.3%) lambing. The per day incidence rate of death in each of the three components of the peri-

parturient period (i.e., before, at, after lambing) was 0.05%, 0.04% and 0.03%, respectively (P = 0.001). Pregnancy toxaemia was the pathological condition most frequently associated with death during the peri-parturient period (41.4% of all deaths, 60.7% of deaths before lambing). Other pathological conditions were ruminal acidosis (11.6% of all deaths, 17.0% of deaths before lambing), genital tract problems (11.6% of all deaths, 44.2% of deaths after lambing), acute clinical mastitis (10.6% of all deaths, 40.4% of deaths after lambing), respiratory infection (9.6% of all deaths, 14.1% of deaths before lambing) and dystocia (5.6% of all deaths, 100.0% of deaths at lambing). Of the 11 ewes that died in association with dystocia, 6 (54.5%) were primigravidae. The proportion of primigravidae ewes that died at lambing (54.5% of those that died at that stage) was significantly higher than of similarly-aged ewes that died before or after lambing (8.0%) (P < 0.0001). With regard to the occurrence of death during the peri-parturient period, in the multivariable analysis, only the average lambing rate at flock level (> 1.50 lambs per ewe) emerged to be significant (P = 0.035). With regard to the occurrence of death before or after lambing, the season during which lambing took place and the number of animals in the flock, respectively, were found to be significant.

The conclusions from the results of the present thesis are summarised herebelow.

(a) Death during the peri-parturient period was found to occur sporadically and most cases occurred 10 days prior to lambing.

(b) Pregnancy toxaemia was the pathological condition most frequently associated with peri-parturient mortality.

(c) Factors found to have a significant association with occurrence of peri-parturient mortality included the average lambing rate at flock level (increased risk with average lambing rate > 1.5), the season of the year during which the lambing period took place (highest risk during the winter), and the number of animals in the flock (highest risk in the flock with ≤ 200 ewes).

Publications associated with the present thesis

The following scientific papers presenting facets of the present thesis, are available:

I. A.P. Politis, N.G.C. Vasieliou, P.J. Cripps, D.V. Liagka, P.T. Boufis, I. Valasi, V.S. Mavrogianni, G.C. Fthenakis (2021) "Mortality of dairy sheep during the peri-parturient period: results of a field investigation in Greece" *Animals*, 11:2172.

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Assistant professor G.A. Gougoulis	Faculty of Veterinary Medicine, University of Thessaly
Assistant professor N.G.C. Vasileiou	Faculty of Animal Science, University of Thessaly

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CHAPTER I

GENERAL INTRODUCTION AND

REVIEW OF THE LITERATURE

A. GENERAL INTRODUCTION

Preface - Objectives of the thesis

The peri-parturient period has been recognised as a particularly dangerous period in the life of animals.

Despite that, there are very little data regarding the occurrence of death of ewes during the peri-parturient period, a problem initially described by Hindson and Winter (1990). More recently, Mavrogianni and Brozos (2008) classified these cases of death according to the time in relation to lambing (before lambing, at lambing or after lambing) and provided a detailed guide for the diagnostic approach that should be followed in such circumstances. Nevertheless, no detailed data are available internationally regarding the incidence, the time of death in relation to lambing and the factors potentially associated with the occurrence of death. Such data would allow a better appraisal of the problem and would be useful for improving health management of sheep flocks.

In cows, there is an increased risk of death and culling due to serious disease during that period (Gibney et al. 2020). Similar findings have been reported for sows, animals in which peri-parturient diseases (lactational failure, genital tract disorders) were found to account for the loss of approximately 25% of adult pigs (Karg and Bilkei 2002), as well as for mares, animals in which peri-parturient haemorrhaging is a significant cause of illness and death (Arnold et al. 2008). In women, incidence of death during the peri-parturient period can be as high as 0.45% in some countries (Wollast et al. 1993), with haemorrhage, hypertensive disorders, abortion and sepsis considered to be the main clinical conditions associated with death (Khan et al. 2006).

Objectives of the thesis

The general objective of the thesis was the advancement of knowledge about the mortality of ewes during the peri-parturient period.

Specific objectives of the thesis were as follows.

- The investigation of the incidence risk of peri-parturient mortality in a field investigation in flocks in Greece.
- The study of the time that such mortality occurs in relation to lambing.
- The identification of the pathological conditions more frequently associated with the death.

- The evaluation of factors potentially associated with death during the peri-parturient period.

The present thesis has been carried out at the Department of Obstetrics and Reproduction of the Veterinary Faculty of the University of Thessaly. During the clinical phase of the work, a large field investigation was performed in 60 flocks in the region of Western Greece during two calendar years. The field part of the work started in the summer of 2017 and was carried out until the autumn of 2019; it was followed by analysis of results and writing up of the thesis.

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Ευχαριστώ την αναπληρώτρια καθηγήτρια του Τμήματος Κτηνιατρικής του Πανεπιστημίου Θεσσαλίας κ. Βάσια Σ. Μαυρογιάννη, μέλος της τριμελούς συμβουλευτικής επιτροπής για την ιδιαίτερα σημαντική βοήθεια της στο σχεδιασμό της μελέτης, στην εκπαίδευσή μου και στην ερμηνεία των νεκροτομικών ευρημάτων. Η ουσιαστική υποστήριξη της σε όλα τα στάδια ήταν ιδιαίτερα σημαντική. Επίσης ευχαριστώ ιδιαίτερα την αναπληρώτρια καθηγήτρια του Τμήματος Κτηνιατρικής του Πανεπιστημίου Θεσσαλίας κ. Ειρήνη Βαλάση, επίσης μέλος της τριμελούς συμβουλευτικής επιτροπής, για την συμβολή της και για τα εποικοδομητικά σχόλια στο τελικό κείμενο της διατριβής.

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Στον καθηγητή της Κτηνιατρικής Σχολής του Πανεπιστημίου Liverpool κ. Peter Cripps, για την συμβολή του και τις πολύτιμες και εύστοχες παρατηρήσεις του στη στατιστική ανάλυση των αποτελεσμάτων.

Στην επίκουρη καθηγήτρια του τμήματος Επιστήμης Ζωικής Παραγωγής του Πανεπιστημίου Θεσσαλίας κ. Ναταλία Βασιλείου για την ουσιαστική βοήθειά της κατά την διάρκεια της μελέτης και την καθοδήγηση της για την καταγραφή των αναλύσεων και των αποτελεσμάτων.

Στη συνάδελφο υποψήφια διδάκτορα κ. Δήμητρα Λιάγκα για την πολύτιμη βοήθεια της στην αναζήτηση της βιβλιογραφίας.

Σε όλα τα μέλη της επιστημονικής ομάδας μας, που αποτελείται από εξαίρετους συναδέλφους, για όλη αυτή την παράλληλη πορεία των τελευταίων ετών.
Στους κτηνοτρόφους, οι οποίοι πρόθυμα συντέλεσαν στην έρευνα αυτή, ανοίγοντας την πόρτα των εκτροφών τους - πάντα είναι εκεί, για να βοηθήσουν στη βελτίωση της δουλειάς τους ως άψογοι επαγγελματίες και τους εκτιμώ ξεχωριστά για αυτό.

Ευχαριστώ τη σύζυγό μου Χριστίνα και τα παιδιά μας Πάνο και Ηλιάνα, που παραμένουν πάντα δίπλα μου δείχνοντας υπομονή και κατανόηση. Η πίστη τους και η υποστήριξή τους κατά τη διάρκεια της εκπόνησης αυτής της διατριβής και της συνολικής προσπάθειας είναι τόσο ζηλευτή όσο και συγκινητική.

Τέλος, ως πράξη ευγνωμοσύνης, ευχαριστώ ιδιαίτερα τους γονείς μου Παναγιώτη και Γαρυφαλλιά και τον αδελφό μου Κωνσταντίνο, στους οποίους οφείλω συνολικά ό,τι είμαι και ό,τι έχω καταφέρει μέχρι σήμερα.

B. DEATH OF EWES DURING THE PERI-PARTURIENT PERIOD

Introduction

There is a clear scarcity of knowledge regarding death of ewes during the peri-parturient period.

In general, the accepted annual incidence of cases of death (by all causes) in sheep flocks is up to 2.0% (Hindson and Winter 2000). In cases of extremely adverse weather conditions, higher incidence has been reported; for example, increased annual ewe mortality, up to 5.4%, has been reported in Australia during a period with higher than average rainfall (Watt et al. 2021), whilst in hill flocks in Scotland annual losses up to 10.0% may occasionally occur (Hindson and Winter 2000).

There is a consensus that the majority of cases of death of adult ewes occur around the lambing period (Scott 2005, Hindson and Winter 2007), although this has not been accurately measured. Bush et al. (2006) have considered the various problems during the peri-parturient period as the most common causes of death in adult ewes.

The cases of death during the peri-parturient period in sheep flocks can be grouped in three components according to the time of death in relation to the time of lambing, i.e., 'pre-partum', 'at lambing' and 'immediately post-partum' (Mavrogianni and Brozos 2008). These authors also underlined the particular financial importance of deaths during the peri-parturient period (Mavrogianni and Brozos 2008).

Based on clinical experience, the major pathological conditions considered to be associated with death of ewes during the peri-parturient period are listed in Table I.i.

Table I.i. Pathological conditions considered (from clinical experience) to be associated with death of ewes during the peri-parturient period.

	Pre-partum	During lambing	Immediately post-partum
Metabolic diseases	Pregnancy toxaemia, hypocalcaemia		Post-partum ketosis, hypocalcaemia
Bacterial systematic diseases	Clostridial infections	Clostridial infections	Clostridial infections
Reproductive diseases		Dystocia (incomplete dilatation of the cervix, uterine / vaginal rupture)	Metritis, uterine / vaginal rupture, clinical mastitis

Moreover, pathological conditions unrelated to reproductive diseases may also lead to death of ewes (e.g., pneumonia), as the result of the immunosuppression (Mavrogianni and Brozos 2008) present during that period. During the peri-parturient period, increased nutritional demands are essential for the animal and may lead to lower availability of nutrients for the immune function of the animals (Coop and Kyriazakis 1999), thus leading to a transient immunocomprise in ewes.

Pathological conditions causing death of ewes before lambing

Fatality rate of ewes with pregnancy toxaemia can be up to 80.0%, especially if animals would remain untreated (Rook 2000). Also, ewes may die, despite treatment (Lima et al. 2012), if its initiation was late. In animals with anorexia, recumbency, concurrent hypocalcaemia and / or neurological signs, the prognosis is poor (Lima et al. 2016) and euthanasia of the affected ewes should be considered for welfare reasons. Based on results of biochemical findings (increased plasma lactate, pyruvate and urea nitrogen concentrations in blood, as well as increased creatinine kinase and aspartate aminotransferase activities in blood), it has been suggested that death in animals with pregnancy toxaemia, would occur as the result of hepatorenal dysfunction (Barakat et al. 2007).

The incidence rate of hypocalcaemia in ewes is considered to be generally less than 5.0%, although it can reach 30.0% under some circumstances. If untreated, the disease can be fatal (Sykes 2007), with the fatality rate rising up to 20.0% (Kaushal and Sharma 2004). Wilkens, Breves et al (2014) and Wilkens, Liesegang et al. (2014) have highlighted differences in homeostatic mechanisms between sheep and goats, which can lead to differing clinical manifestation and severity of the disease. Oetzel (1988) postulated that sheep were more likely to develop hypocalcaemia in late pregnancy, possibly due to the relatively larger foetoplacental complex, rather than goats, which had a relatively higher milk production, and, thus, could develop the disease during early lactation (Oetzel 1988).

Pathological conditions causing death of ewes at lambing

Various obstetrical problems can occur in ewes. In Australia, sheep farmers reported that death during lambing were the most frequent cases of death in ewes (Watt et al. 2021).

A primary cause of dystocia in ewes is the incomplete dilatation of the cervix (Mavrogianni 2007). Other usual causes of dystocia include malpresentation, abnormal position / posture of the foetus or oversized - multiple foetuses (Hindson and Winter 2007). Other problems (e.g., uterine torsion, hydrallantois, vaginal / uterine rupture) occur less often (Hindson and Winter 2007).

In most cases, if diagnosis is made early and veterinary intervention takes place timely, treatment can be successful (Ijaz and Talafha 1999). In fact, Scott (1989, 2005) reported a 97% survival rate of ewes after on-farm caesarian operations that were performed timely; based on that figure, he calculated that approximately 400,000 ewes could be saved annually in the United Kingdom, if veterinary intervention would be applied at the early stages of a dystocia problem. The general health of the ewe would also play an important role in the outcome of such problems and ewes with a good general health were found to have a lower mortality rate in such cases (Rahim and Arthur 1982).

Fatal implications of dystocia are mainly the result of late intervention, inappropriate handling of the animals or post-surgery infections and may account for up to 10% of cases (Sobiraj 1994, Kloss et al. 2002, Ennen et al. 2013). Increased fatality rate has been recorded in dystocias occurring in animals during the first two lambings in their life, in animals during the winter and in animals bearing three foetuses (Majeed 1994).

In general, the prognosis is bad in cases complicated with other pathological conditions (e.g., rupture of the bladder, pregnancy toxaemia) (Mosdol 1999). Incorrect management of such pathological conditions would predispose to death of the affected ewes (Hindson and Winter 2000).

Pathological conditions causing death of ewes after lambing

Various problems of the genital tract can occur after lambing and these are often associated with dystocia.

Voigt et al. (2021) reported various complications of caesarean section in ewes; these included peritonitis, liver damage, metritis, septicaemia, vaginal rupture and damage to the urinary tract. Incidence of death in such cases could be up to 3.8%, but it could increase up to nine times if there were preexisting pathological conditions, e.g., pregnancy toxaemia, peritonitis, uterine torsion.

Other relevant studies were performed by Waage and Wangensteen (2013), who reported 1.2% fatality rate of ewes post-partum subsequently to dystocia, usually because of peritonitis or uterine rupture, Brounts et al. (2004), who reported 19.0% fatality rate of ewes post-partum subsequently to dystocia coupled with pregnancy toxaemia, and Scott (1989), who reported 2.2%

fatality rate of ewes post-partum subsequently to dystocia when foetuses were alive or had died only early before the obstetrical manipulation versus 42.9% fatality rate when foetuses had autolysis and emphysema.

Hypomagnesaemia may also occur post-partum, with the fatality rate reaching 5.0% and occasionally up to 20.0%, if animals were left untreated (Herd 1966, Friend et al. 2020).

Finally, acute clinical mastitis can occur immediately after lambing and may potentially lead to death of affected ewes (Gelasakis et al. 2015).

Principles of diagnosis of the problems

In every case of investigation into a flock with increased peri-parturient losses, one should always take into account information regarding the history and the health management protocols of the flock. Further, information regarding the individual animals that died (e.g., body condition, clinical signs, previous therapeutic regimens and their efficacy, time of death related to parturition) should be collected (Hindson and Winter, 2000). Time of death in relation to parturition can easily be determined by post-mortem examination of the uterus, during which its condition and the presence / absence of foetus(-es) can be confirmed.

A detailed post-mortem examination of the ewe(s) that died is helpful to classify cases of death into three different categories: (a) related to pregnancy, parturition or the puerperium, (b) caused by other disease, but precipitated by the stress of pregnancy or parturition and (c) totally unrelated to pregnancy or parturition (Hindson and Winter 2000).

CHAPTER II

FIELD INVESTIGATION OF MORTALITY OF EWES IN GREECE DURING THE PERI-PARTURIENT PERIOD

A. MATERIALS AND METHODS

Flocks and investigation procedures

Sixty (60) dairy sheep flocks were included in the study, which was performed during two consecutive years, the first from August 2017 to July 2018 and the second from August 2018 to July 2019.

In total, there were 13,418 and 13,599 ewes at the start of each of the two study years, respectively, in the 60 flocks. In accordance with the national procedures for animal identification, all ewes bore plastic ear-tags in both ears with a unique national registry number. All the flocks were located in the administrative region of Western Greece and were maintained for dairy production; in most flocks, lambs were weaned at 45 to 60 days (although occasionally early weaning occurred at 20–25 days) and then ewes were milked for a period of 6 to 8 months. Flocks in the study came from small-sized family farms to large commercial farms, with 50 to 812 ewes. Data regarding management of the flocks, available as part of the standard monitoring and veterinary care of the flocks were considered (Table II.i and II.ii).

Table II.i. Variables (n = 15; flock-level factors) evaluated for potential association with death during the peri-parturient period in 60 sheep flocks in Greece.

Variables (n = 15)
Management system applied in the flock intensive / semi-intensive / semi-extensive or extensive (description according to the EFSA classification[1])
Season of the year during which the lambing period took place autumn / winter / spring / summer
Number of animals in the flock no.
Breed of ewes Greek pure-breed animals / imported pure-breed animals / crossbred animals
Average lambing rate at flock level rate
Control or reproduction (yes, with intravaginal use of progestogen sponges / yes, with administration of melatonin implants / no
Feed origin homemade / commercial
Inclusion of roughage into the diet yes / no

Table II.i. (continued).

Variables (n = 15)
Intramammary administration of antibiotics at the end of the previous lactation period yes / no
Nutritional modifications performed during the last stage of pregnancy yes / no
Administration of anthelmintics at the end of pregnancy yes / no
Administration of anti-clostridial vaccination at the end of pregnancy yes / no
Administration of anti-mastitis vaccination at the end of pregnancy yes / no
Experience of the farmer years
Presence of working staff in the farm yes / no

1. European Food Safety Authority (2014).

Table II.ii. Characteristics of the variables (n = 15; flock-level factors) evaluated for potential association with death during the peri-parturient period in 60 sheep flocks in Greece.

Variables (n = 15)			
Management system applied in the flock[1]			
Intensive	Semi-intensive	Semi-extensive or extensive	
n = 13	n = 12	n = 35	
Season of the year during which the lambing period took place			
Autumn	Winter	Spring	Summer
n = 35	n = 5	n = 0	n = 19
Number of animals in the flock			
≤ 200 ewes	200–500 ewes	> 500 ewes	
n = 38	n = 17	n = 5	
Breed of ewes			
Greek pure-breed animals	Imported pure-breed animals	Crossbred animals	
n = 15	n = 18	n = 27	
Average lambing rate at flock level			
≤ 1.5		> 1.5	
n = 45		n = 15	

Table II.ii. (continued).

Variables (n = 15)		
Control of reproduction		
Yes, with intravaginal use of progestogen sponges	Yes, with administration of melatonin implants	No
n = 13	n = 9	n = 38
Feed origin		
Homemade		Commercial
n = 26		n = 34
Inclusion of roughage into the diet		
Yes		No
n = 58		n = 2
Intramammary administration of antibiotics at the end of the previous lactation period		
Yes		No
n = 21		n = 39
Nutritional modifications performed during the last stage of pregnancy		
Yes		No
n = 28		n = 32
Administration of anthelmintics at the end of pregnancy		
Yes		No
n = 60		n = 0
Administration of anti-clostridial vaccination at the end of pregnancy		
Yes		No
n = 60		n = 0
Administration of anti-mastitis vaccination at the end of pregnancy		
Yes		No
n = 25		n = 35
Experience of the farmer		
0–10 years	11–20 years	> 20 years
n = 13	n = 30	n = 17

Table II.ii. (continued).

Variables (n = 15)	
Presence of working staff in the farm	
Yes	No
n = 39	n = 21

1. European Food Safety Authority (2014).

During the study period, all cases of death in each of the flocks were recorded. All flocks were under veterinary care and were visited regularly (every 3 to 4 days during the lambing period and once fortnightly otherwise), as well as outside that schedule in cases of emergency.

At the individual animal level, the peri-parturient period was defined from 10 days before to 7 days after lambing (i.e., a total of 18 days). In all the ewes that died during that period, as reported by the respective farmers, a detailed post-mortem examination was performed. The time of death in relation to lambing was established.

For this, the farmer provided the initial relevant information, which was subsequently confirmed by a post-mortem examination of the genital tract (Hindson and Winter 1990). If the cervix was closed and the uterus was gravid, then the ewe was considered to have died before lambing. If the cervix was open with presence of a dead embryo or fetal remains in the uterus or the genital tract, then death was considered to have occurred at the time of lambing. In the absence of embryo(s) within the uterus, the ewe was considered to have recently lambed or aborted; in that case, the cervix could be found open or, after the third to fourth day after lambing, closed, although material indicative of a recent gestation would have been evident in the uterus (Ioannidi et al. 2020).

During the post-mortem examination, the following were evaluated, as recommended by Mavrogianni and Brozos (2008).

- If the ewe died before lambing: body condition, liver, perirenal and peritoneal fat, urine (sampling for measurement of ketone bodies), aqueous humour (sampling for measurement of calcium [only within 24 h of death]), placenta and condition of the foetus(es), and other organs.
- If the ewe died at lambing: opening of the cervix, foetus(es), placenta, genital tract, other organs, and aqueous humour (sampling for measurement of calcium [only within 24 h of death]).
- If the ewe died after lambing: genital tract, subcutaneous tissues, aqueous humour (sampling for measurement of calcium [only within 24 h of death]), mammary glands, and other organs.

Following the post-mortem examination and based on its results, relevant and appropriate examinations were also performed in the other animals of the flock and appropriate samples were collected, in order to establish a diagnosis of the prevailing pathological condition at flock level. Finally, a diagnosis was made of the pathological condition in the ewe.

Data management and analysis

Data were entered into Microsoft Excel and analyzed using SPSS v. 21 (IBM Analytics, Armonk, NY, USA). A basic descriptive analysis was performed and exact binomial confidence intervals (CIs) were obtained.

In relation to the time of occurrence within each year of the study, cases of death were classified as having occurred (a) before, (b) during or (c) after the peri-parturient period.

Cases of death within the 18-day long peri-parturient period were classified as having occurred (a) before lambing, (b) at lambing or (c) after lambing by applying the diagnostic criteria presented above.

For estimating the incidence rates and risks, the exact number of ewes that entered each study year, and the exact number of cases of death were known. The number of animals that left the flock (i.e., culled or sold) was believed to be very low (< 1%), but it was not recorded or included in the calculations. Incidence rates and risks for any time period were calculated by subtracting the cumulative sum of ewes that had died before that time period from the number that had started the year. The annual incidence risk of death or of death during the peri-parturient period for each year of the study was defined as the proportion of ewes that died among those at risk. Then, an average incidence risk was calculated as the mean of the two annual incidence risks obtained. The incidence rates death in each of the three components of the peri-parturient period (before, at, after lambing) were compared after taking into account the different lengths of each of these three components (i.e., 10, 1, 7 days, respectively, for a total of 18 days) by using a Poisson regression for comparison of events per sheep-day at risk.

Fifteen (15) variables were evaluated for potential association with death during the peri-parturient period (Table II.i). For each of these variables, categories were created according to the farmers' answers. The outcomes of 'death before / at / after lambing' were considered. Exact binomial CIs were obtained. Initially, the importance of predictors was assessed by using cross-tabulation with Pearson's chi-square test and with simple logistic regression without random effects.

Subsequently, multivariable models were created using mixed-effects logistic regression with flocks as the random effect, and initially offering to the model all variables, which achieved a significance of $P < 0.2$ in the univariable analysis (Table II.iii.). Variables were removed from the initial model by backwards elimination. The *P* value of removal of a variable was assessed by the likelihood ratio test, and for those with a *P* value of > 0.2 the variable with the largest probability was removed. This process was repeated until no variable could be removed with a *P* value of > 0.2. The variables required for the final multivariable model in each occasion are shown in Table II.iii.

After performing the analyses as detailed above with the results of both years of the study considered together, the analyses were repeated with the results of each year of the study considered separately.

Finally, the outcomes 'occurrence of death during the peri-parturient period in at least one year of the study' and 'occurrence of death during the peri-parturient period both years of the study' (i.e., independently of the time of death in relation to lambing) were considered. The above methodology was also employed for these analyses. The variables required for the final multivariable model for each occasion are also shown in Table II.iii.

Table II.iii. Details of multivariable models employed for the evaluation of the occurrence of death during the peri-parturient period in sheep flocks in Greece.

Outcome	Variables offered to the multivariable models (n)	Variables required in the final models
Death before lambing	5	(a) season of the year during which the lambing period took place, (b) number of animals in the flock
Death at lambing	4	(a) breed of ewes, (b) presence of working staff in the farm
Death after lambing	6	(a) number of animals in the flock, (b) breed of ewes, (c) control or reproduction, (d) nutritional modifications performed during the last stage of pregnancy, (e) experience of the farmer
Occurrence of death during the peri-parturient period in at least one year of the study	7	(a) season of the year during which the lambing period took place, (b) average lambing rate at flock level, (c) inclusion of roughage into the diet
Occurrence of death during the peri-parturient period in both years of the study	5	(a) experience of the farmer, (b) presence of working staff in the farm

In all analyses, statistical significance was defined at $P < 0.05$.

B. RESULTS

Incidence risk of death during the peri-parturient period in the sheep flocks

A total of 345 deaths of adult ewes were recorded during the two years of the study. Specifically, 160 deaths occurred in the first and 185 deaths in the second year, and the respective annual incidence risks were 1.2% (95% CI: 1.0%–1.4%) and 1.4% (95% CI: 1.2%–1.6%) (P = 0.22 between the two years); hence, the average incidence risk during the two years was 1.3%. There were 147 deaths outside the peri-parturient period: 79 before and 68 after it (P > 0.30 for all comparisons between deaths before or after the period and between years of the study).

A total of 198 deaths (57.4% of all deaths, 95% CI: 52.1%–62.5%) occurred during the peri-parturient period. Specifically, 89 deaths occurred in the first and 109 deaths in the second year of the study and the respective annual incidence risks were 0.7% (95% CI: 0.6%–0.8%) and 0.8% (95% CI: 0.7%–1.0%) (P = 0.18 between the two years); hence, the average incidence risk during the two years was 0.7% (P < 0.0001 compared to deaths that occurred outside the peri-parturient period) (Table II.iv).

Table II.iv. Frequency and incidence risk of cases of death of adult ewes during a two-year study in 60 sheep flocks in Greece.

1st year of the study				2nd year of the study				Both years	
Ewes	Death before the pp period[1]	Death during the pp period	Death after the pp period	Ewes	Death before the pp period	Death during the pp period	Death after the pp period	All deaths	Death during the pp period
				n					
13,418	40	89	31	13,599	39	109	37	345	198
				Incidence risks					
	0.3%[a]	0.7%[a,b]	0.2%[b]		0.3%[c]	0.8%[c,d]	0.3%[d]	1.3%	0.7%

1. pp: peri-parturient period.
[a–d] P < 0.001 for comparisons between incidence risks with similar superscripts.

Among the flocks, the average incidence risk of death during the peri-parturient period varied from 0.0% to 6.0% during the two years (P < 0.0001 between flocks) (Table II.v). It varied from 0.0% to 8.8% in the first and 0.0% to 9.2% in the second year of the study. Death during the peri-parturient

period were recorded in a total of 28 flocks (46.7%, 95% CI: 34.6%–59.1%). There was no significant difference in the proportion of flocks, in which cases of death during the peri-parturient period were recorded in each year of the study: 25.0% (95% CI: 15.8%–37.2%) and 38.3% (95% CI: 27.1%–51.0%), respectively (P = 0.12), although in 10 flocks (16.7, 95% CI: 9.3%–28.0%) cases of deaths were recorded during both years.

Table II.v. Incidence risk of cases of death during the peri-parturient period in each of 60 sheep flocks during a two-year study in Greece.

Flock reference no.	1st year of the study	2nd year of the study	Both years
1	1.32%	0.00%	0.66%
2	2.00%	5.00%	3.50%
3	0.00%	0.00%	0.00%
4	0.00%	0.00%	0.00%
5	0.00%	0.00%	0.00%
6	0.00%	1.80%	0.90%
7	0.00%	0.75%	0.38%
8	3.35%	3.28%	3.32%
9	0.00%	0.00%	0.00%
10	0.00%	0.00%	0.00%
11	0.00%	0.00%	0.00%
12	0.00%	0.00%	0.00%
13	0.00%	1.56%	0.78%
14	0.00%	0.00%	0.00%
15	0.00%	0.00%	0.00%
16	0.00%	0.00%	0.00%
17	0.00%	0.00%	0.00%
18	0.00%	0.95%	0.47%
19	0.00%	0.00%	0.00%
20	0.00%	0.00%	0.00%
21	1.04%	1.02%	1.03%
22	0.00%	1.31%	0.65%

Table II.v. (continued).

Farm reference no.	1st year of the study	2nd year of the study	Both years
23	0.00%	2.63%	1.32%
24	0.00%	4.08%	2.04%
25	1.47%	0.47%	0.97%
26	1.33%	2.07%	1.70%
27	0.00%	0.00%	0.00%
28	3.00%	0.00%	1.50%
29	0.00%	0.00%	0.00%
30	0.00%	1.14%	0.57%
31	6.29%	0.00%	3.15%
32	0.00%	0.00%	0.00%
33	0.00%	0.00%	0.00%
34	0.00%	0.00%	0.00%
35	0.00%	0.00%	0.00%
36	7.22%	0.00%	3.61%
37	0.00%	0.00%	0.00%
38	0.00%	0.00%	0.00%
39	0.00%	0.00%	0.00%
40	0.00%	0.00%	0.00%
41	3.39%	3.13%	3.26%
42	0.00%	0.00%	0.00%
43	1.83%	2.28%	2.06%
44	0.00%	1.49%	0.74%
45	0.00%	0.00%	0.00%
46	0.00%	0.00%	0.00%
47	0.00%	0.00%	0.00%
48	0.00%	0.00%	0.00%
49	0.00%	4.46%	2.23%
50	0.00%	0.00%	0.00%

Table II.v. (continued).

Farm reference no.	1st year of the study	2nd year of the study	Both years
51	8.84%	3.19%	6.01%
52	6.47%	2.87%	4.67%
53	0.00%	3.90%	1.95%
54	0.00%	0.00%	0.00%
55	0.00%	1.31%	0.65%
56	2.44%	0.00%	1.22%
57	0.00%	9.17%	4.58%
58	1.23%	1.75%	1.49%
59	0.00%	0.00%	0.00%
60	0.00%	0.00%	0.00%

Time of death in relation to lambing

Of the 198 deaths during the peri-parturient period, most (n = 135; 68.2%, 95% CI: 61.4%–74.3%) occurred before lambing. Fewer cases of death occurred at (n = 11; 5.6%, 95% CI: 3.1%–9.7%) or after (n = 52; 26.3%, 95% CI: 20.6%–32.8%) lambing. There was no significant difference in these proportions between the two years of the study ($P > 0.10$ for all comparisons) (Table II.vi).

The per day incidence rate of death in each of the three components of the peri-parturient period (i.e., before, at, after lambing) was 0.05%, 0.04% and 0.03%, respectively ($P = 0.001$ for the overall difference between the three components considered together, $P < 0.001$ for the difference in the incidence rate before and after lambing, $P > 0.23$ for the other comparisons) (Table II.vii, Figure II.1).

Cases of death before, at, and after lambing were recorded in 24 (40.0%), 5 (8.3%) and 20 (33.3%) flocks, respectively ($P = 0.0002$). In 3 flocks (5.0%, 95% CI: 1.7–13.7%), deaths were recorded during the same year in ewes before, at and after lambing.

Table II.vi. Frequency[1] of death during the peri-parturient period in relation to each of the three components of the period, during a two-year study in 60 sheep flocks in Greece.

1st year of the study			2nd year of the study			Both years		
Components of the peri-parturient period (time of death in relation to lambing)								
Before	At	After	Before	At	After	Before	At	After
n								
Total = 89			Total = 109			Total = 198		
65	4	20	70	7	32	135	11	52
Proportion of cases of death during the peri-parturient period within the respective component of the peri-parturient period								
73.0%	4.5%	22.5%	64.2%	6.4%	29.4%	68.2%	5.5%	26.3%

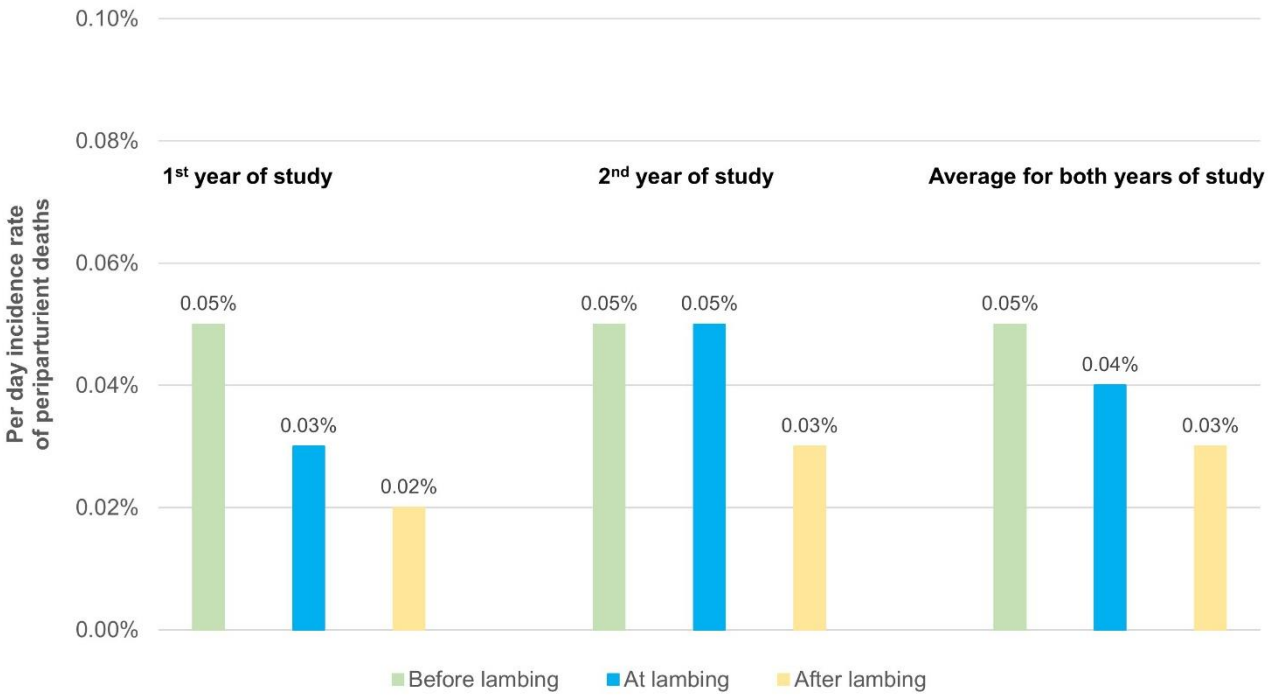
1. The figures refer only to cases of death that occurred within the 18-day long peri-parturient period.

Table II.vii. Incidence rate of death during each of the three components of the peri-parturient period[1] during a two-year study in 60 sheep flocks in Greece (Poisson regression).

Components of the peri-parturient period	Incidence rate[2]	Incidence rate ratios[3] (95% confidence intervals)	*P* value
			0.001
Before lambing (duration: 10 days)	0.05%	1.807 (1.313-2.489)	< 0.001
At lambing (duration: 1 day)	0.04%	1.480 (0.772–2.837)	0.237
After lambing (duration: 7 days)	0.03%	reference	

1. These cases of death refer only to the 18-day long peri-parturient period.
2. Incidence rate per sheep-day at risk.
3. Incidence rate ratios calculated against the lowest incidence rate.

Figure II.1. Incidence rate per sheep-day at risk of death during the peri-parturient period, during a two-year study in 60 sheep flocks in Greece in relation to the time of lambing.

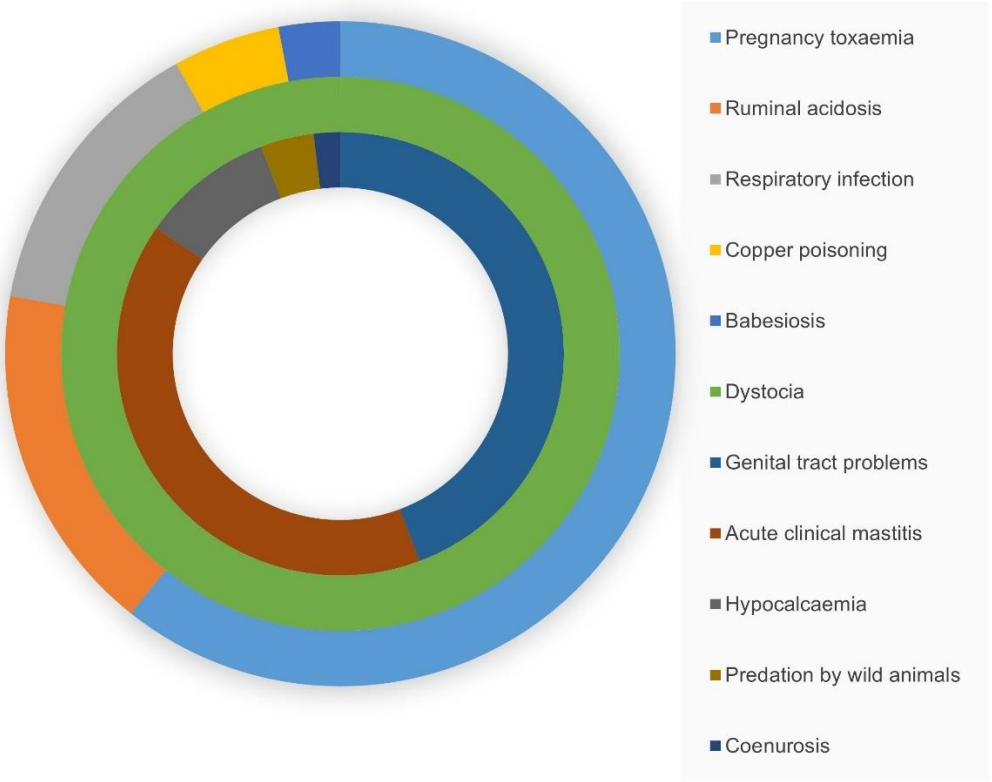


Green bars: before lambing (duration: 10 days); turquoise bars: at lambing (duration: 1 day); yellow bars: after lambing (duration: 7 days).

Pathological conditions associated with death during the peri-parturient period

Pregnancy toxaemia was the pathological condition most frequently associated with death during the peri-parturient period (n = 82; 41.4% of all deaths, 60.7% of deaths before lambing). Other pathological conditions were ruminal acidosis (n = 23; 11.6% of all deaths, 17.0% of deaths before lambing), genital tract problems (including genital injury, uterine prolapse, rectal prolapse, metritis) (n = 23; 11.6% of all deaths, 44.2% of deaths after lambing), acute clinical mastitis (n = 21; 10.6% of all deaths, 40.4% of deaths after lambing), respiratory infection (n = 19; 9.6% of all deaths, 14.1% of deaths before lambing) and dystocia (n = 11; 5.6% of all deaths, 100.0% of deaths at lambing) (Figure 2, Table II.viii). There was no significant difference between the two years of the study in the proportion of the various pathological conditions associated with such deaths (P = 0.27).

Figure II.2. Ring-pie of the relative frequency of pathological conditions associated with death in relation during the peri-parturient period to the time of lambing.



Outer ring: pathological conditions associated with death before lambing (duration: 10 days); middle ring: pathological conditions associated with death at lambing (duration: 1 day); inner ring: pathological conditions associated with death after lambing (duration: 7 days).
Colours in each ring indicate the pathological conditions associated with death during the peri-parturient period.

Table II.viii. Frequency[1] of pathological conditions associated with death during the peri-parturient period, in relation to each of the three components of that period during a two-year study in 60 sheep flocks in Greece.

Pathological condition	Components of the peri-parturient period		
	Before lambing	At lambing	After lambing
Pregnancy toxaemia	82		
Ruminal acidosis	23		
Genital tract problems (genital injury, uterine prolapse, rectal prolapse, metritis)			23
Acute clinical mastitis			21
Respiratory infections	19		

Table II.viii. (continued).

Pathological condition	Components of the peri-parturient period		
	Before lambing	At lambing	After lambing
Dystocia		11	
Copper poisoning	7		
Hypocalcaemia			5
Babesiosis	4		
Predation by wild animals			2
Coenurosis			1
Total	135	11	52

1. The figures refer only to cases of death that occurred within the 18-day long peri-parturient period.

Table II.ix presents the numbers of flocks with various pathological conditions associated with cases of death during the peri-parturient period. Again, pregnancy toxaemia occurred in more flocks than any other pathological condition. Cases of death associated with pregnancy toxaemia were recorded in 19 flocks; in 15 (78.9%) of these flocks, cases of death during the peri-parturient period associated with ruminal acidosis, dystocia, genital tract problems or acute clinical mastitis were also found.

Further, in 6 of the 7 flocks (85.7%) in which cases of death associated with ruminal acidosis were found, cases of pregnancy toxaemia were also recorded. Moreover, cases of pregnancy toxaemia were recorded in 7 of 11 flocks (63.6%) that had cases of death associated with genital tract problems, in 7 of 11 flocks (63.6%) that had cases of death associated with mastitis, and in 3 of 5 flocks (60.0%) that had cases of death associated with dystocia (P = 0.72).

Table II.ix. Number of flocks in which the pathological conditions associated with cases of death during the peri-parturient period[1], were recorded during a two-year study in 60 sheep flocks in Greece.

Pathological condition	Number (proportion) of flocks	Mean number of ewes that died per flock
Pregnancy toxaemia	19 (31.7%)	4.3
Acute clinical mastitis	11 (18.3%)	1.9
Genital tract problems	11 (18.3%)	2.1
Respiratory infection	7 (11.7%)	2.7

Table II.ix. (continued).

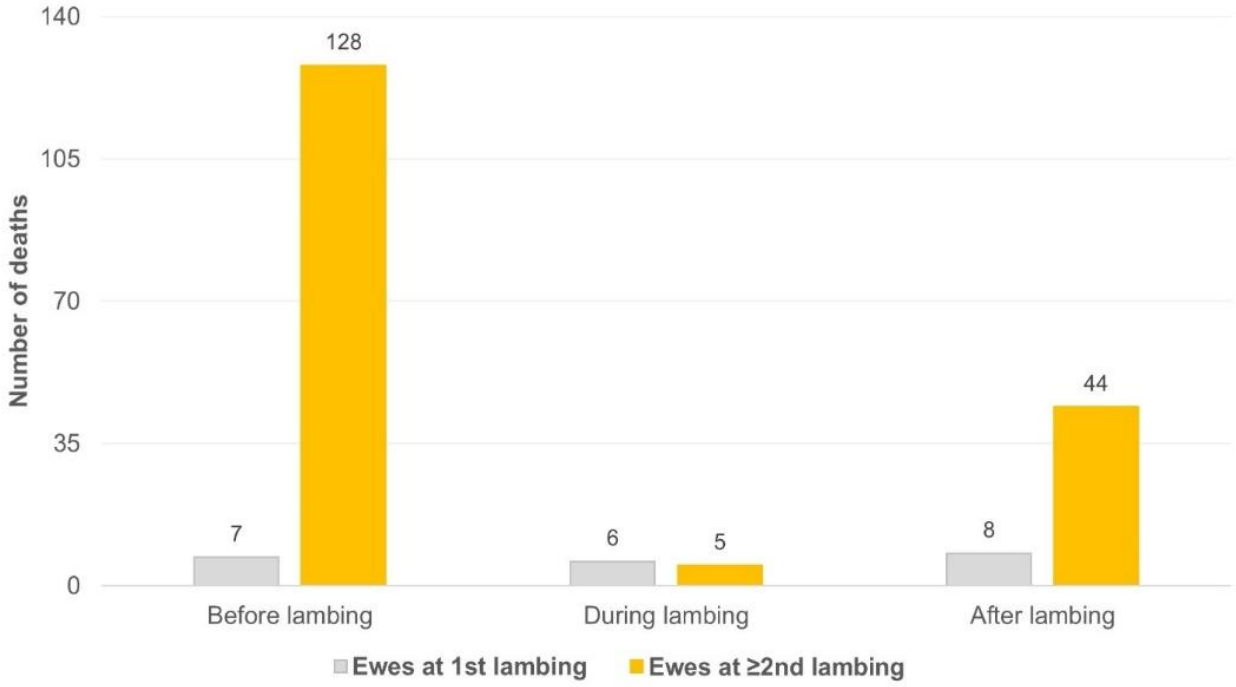
Pathological condition	Number (proportion) of flocks	Mean number of ewes that died per flock
Ruminal acidosis	7 (11.7%)	3.3
Dystocia	5 (8.3%)	2.2
Hypocalcaemia	4 (6.7%)	1.3
Copper poisoning	2 (3.3%)	3.5
Predation by wild animals	2 (3.3%)	1.0
Babesiosis	1 (1.7%)	4.0
Coenurosis	1 (1.7%)	1.0

1. The figures refer only to cases of death that occurred within the 18-day long peri-parturient period.

Age of the ewes that died

Of the 198 ewes that died during the peri-parturient period, 13 were primigravidae (7 died before and 6 at lambing) and 8 were primiparous; that is, 21 ewes (10.6% of all that died) had not lambed before (Figure II.3). Of the 11 ewes that died in association with dystocia, 6 (54.5%) were primigravidae. The proportion of primigravidae ewes that died at lambing (54.5% of those that died at that stage) was significantly higher than of similarly-aged ewes that died before or after lambing (8.0%) ($P < 0.0001$).

Figure II.3. Frequency of cases of death during the peri-parturient period in relation to the component of the peri-parturient period and the age of the ewes that died, during a two-year study in 60 sheep flocks in Greece (gray: ewes at 1st lambing; orange: ewes at ≥ 2nd lambing).



The duration of the period before lambing was 10 days; at lambing, 1 day; after lambing, 7 days.

Factors associated with occurrence of death during the peri-parturient period

Occurrence of death during the peri-parturient period in at at least one year of the study

With regard to the occurrence of death during the peri-parturient period in at at least one year of the study, a significant association was evident with the following two variables during the univariable analysis: the season when the lambing period took place (P = 0.008) and the average lambing rate at flock level (P = 0.017). Among the variables included in the multivariable analysis, the average lambing rate at flock level emerged to be significant (P = 0.035), while a tendency was noted for the season during which the lambing period took place (P = 0.07) (Table II.x).

Table II.x. Results of multivariable analysis for associations with occurrence[1] of death during the peri-parturient period in at least one year of a two-year study in 60 sheep flocks in Greece.

Variable	Proportion	Odds ratio[2] (95% confidence intervals)	*P* value
Average lambing rate at flock level			0.035
≤ 1.5 (n = 45)	37.8%	reference	
> 1.5 (n = 15)	73.3%	4.529 (1.243–16.510)	0.022

1. The figures refer only to cases of deaths that occurred within the 18-day long peri-parturient period.
2. Odds ratio calculated against the lowest prevalence associations of the variables.

Occurrence of death during each of the three components of the peri-parturient period in at at least one year of the study

With regard to occurrence of death during the before lambing component of the peri-parturient period, a significant association was evident with five variables during the univariable analysis (Table II.xi). Among the variables included in the multivariable analysis, only the lambing season emerged to be a significant factor (*P* = 0.002).

Table II.xi. Incidence risk of cases of death of ewes during the 'before lambing' component of the peri-parturient period during a two-year study in 60 sheep farms in Greece, in accord with factors (n = 15) evaluated for potential association with these deaths.

Management system applied in the flock (*P* = 0.002)			
Intensive (n = 13)	Semi-intensive (n = 12)	Semi-extensive or extensive (n = 35)	
0.9%	0.3%	0.5%	
Season of the year during which the lambing period took place (*P* < 0.0001)			
Winter (n = 5)	Spring (n = 0)	Summer (n = 19)	Autumn (n = 36)
1.3%	n/a	0.7%	0.3%
Number of animals in the flock (*P* = 0.034)			
≤ 200 ewes (n = 38)	200 – 500 ewes (n = 17)	> 500 ewes (n = 5)	
0.7%	0.5%	0.3%	

Table II.xi. (continued).

Breed of ewes (P = 0.96)		
Greek pure-breed animals (n = 15)	Imported pure-breed animals (n = 18)	Crossbred animals (n = 27)
0.6%	0.5%	0.5%
Average lambing rate at flock level (P = 0.034)		
≤ 1.5 (n = 45)		> 1.5 (n = 15)
0.5%		0.8%
Control of reproduction (P = 0.026)		
Yes, with intravaginal use of progestogen sponges (n = 13)	Yes, with administration of melatonin implants (n = 9)	No (n = 38)
0.4%	0.5%	0.6%
Feed origin (P = 0.52)		
Homemade (n = 26)		Commercial (n = 34)
0.6%		0.5%
Inclusion of roughage into the diet (P = 0.77)		
Yes (n = 58)		No (n = 2)
0.6%		0.4%
Intramammary administration of antibiotics at the end of the previous lactation period (P = 0.96)		
Yes (n = 21)		No (n = 39)
0.6%		0.5%
Nutritional modifications performed during the last stage of pregnancy (P = 0.39)		
Yes (n = 28)		No (n = 32)
0.5%		0.6%
Administration of anthelmintics at the end of pregnancy		
Yes (n = 60)		No (n = 0)
0.5%		n/a
Administration of anti-clostridial vaccination at the end of pregnancy		
Yes (n = 60)		No (n = 0)
0.5%		n/a

Table II.xi. (continued).

Administration of anti-clostridial vaccination at the end of pregnancy	
Yes (n = 60)	No (n = 0)
0.5%	n/a
Administration of anti-mastitis vaccination at the end of pregnancy (*P* = 0.24)	
Yes (n = 25)	No (n = 35)
0.5%	0.6%

Experience of the farmer (*P* < 0.0001)		
0 – 10 years (n = 13)	11 – 20 years (n = 30)	> 20 years (n = 17)
0.7%	0.3%	1.0%

Presence of working staff in the farm (*P* = 0.52)	
Yes (n = 39)	No (n = 21)
0.6%	0.5%

With regard to occurrence of death during the at lambing component of the peri-parturient period, a significant association was evident with one variable during the univariable analysis (Table II.xii). Among the variables included in the multivariable analysis, none was found to be significant, although a tendency for significance was seen for the lack of working staff in the flock (*P* = 0.06).

Table II.xii. Incidence risk of cases of death of ewes during the 'at lambing' component of the peri-parturient period during a two-year study in 60 sheep farms in Greece, in accord with factors (n = 15) evaluated for potential association with these deaths.

Management system applied in the flock (*P* = 0.093)		
Intensive (n = 13)	Semi-intensive (n = 12)	Semi-extensive or extensive (n = 35)
0.1%	0.0%	0.1%

Season of the year during which the lambing period took place (*P* = 0.08)			
Winter (n = 5)	Spring (n = 0)	Summer (n = 19)	Autumn (n = 36)
0.1%	n/a	1.0%	0.0%

Table II.xii. (continued).

Number of animals in the flock (P = 0.29)		
≤ 200 ewes (n = 38)	200 – 500 ewes (n = 17)	> 500 ewes (n = 5)
0.1%	0.1%	0.0%
Breed of ewes (P = 0.10)		
Greek pure-breed animals (n = 15)	Imported pure-breed animals (n = 18)	Crossbred animals (n = 27)
0.1%	0.1%	0.0%
Average lambing rate at flock level (P = 0.49)		
≤ 1.5 (n = 45)		> 1.5 (n = 15)
0.1%		0.0%
Control of reproduction (P = 0.17)		
Yes, with intravaginal use of progestogen sponges (n = 13)	Yes, with administration of melatonin implants (n = 9)	No (n = 38)
0.0%	0.1%	0.0%
Feed origin (P = 0.40)		
Homemade (n = 26)		Commercial (n = 34)
0.0%		0.1%
Inclusion of roughage into the diet (P = 0.62)		
Yes (n = 58)		No (n = 2)
0.1%		0.0%
Intramammary administration of antibiotics at the end of the previous lactation period (P = 0.38)		
Yes (n = 21)		No (n = 39)
0.1%		0.0%
Nutritional modifications performed during the last stage of pregnancy (P = 0.69)		
Yes (n = 28)		No (n = 32)
0.1%		0.0%
Administration of anthelmintics at the end of pregnancy		
Yes (n = 60)		No (n = 0)
0.5%		n/a

Table II.xii. (continued).

Administration of anti-clostridial vaccination at the end of pregnancy	
Yes (n = 60)	No (n = 0)
0.5%	n/a
Administration of anti-mastitis vaccination at the end of pregnancy (*P* = 0.95)	
Yes (n = 25)	No (n = 35)
0.1%	0.1%

Experience of the farmer (*P* = 0.58)		
0 – 10 years (n = 13)	11 – 20 years (n = 30)	> 20 years (n = 17)
0.0%	0.1%	0.0%

Presence of working staff in the farm (*P* = 0.035)	
Yes (n = 39)	No (n = 21)
0.0%	0.1%

With regard to occurrence of death during the after lambing component of the peri-parturient period, a significant association was evident with three variables during the univariable analysis (Table II.xiii). Among the variables included in the multivariable analysis, only the number of animals in the flock was a significant factor (*P* = 0.024).

Table II.xiii. Incidence risk of cases of death of ewes during the 'after lambing' component of the peri-parturient period during a two-year study in 60 sheep farms in Greece, in accord with factors (n = 15) evaluated for potential association with these deaths.

Management system applied in the flock (*P* = 0.30)		
Intensive (n = 13)	Semi-intensive (n = 12)	Semi-extensive or extensive (n = 35)
0.3%	0.1%	0.2%

Season of the year during which the lambing period took place (*P* = 0.48)			
Winter (n = 5)	Spring (n = 0)	Summer (n = 19)	Autumn (n = 36)
0.3%	n/a	0.3%	0.2%

Table II.xiii. (Continued).

Number of animals in the flock (P = 0.022)		
≤ 200 ewes (n = 38)	200 – 500 ewes (n = 17)	> 500 ewes (n = 5)
0.4%	0.1%	0.1%
Breed of ewes (P = 0.19)		
Greek pure-breed animals (n = 15)	Imported pure-breed animals (n = 18)	Crossbred animals (n = 27)
0.3%	0.1%	0.2%
Average lambing rate at flock level (P = 0.018)		
≤ 1.5 (n = 45)		> 1.5 (n = 15)
0.2%		0.4%
Control of reproduction (P = 0.08)		
Yes, with intravaginal use of progestogen sponges (n = 13)	Yes, with administration of melatonin implants (n = 9)	No (n = 38)
0.2%	0.4%	0.2%
Feed origin (P = 0.70)		
Homemade (n = 26)		Commercial (n = 34)
0.2%		0.2%
Inclusion of roughage into the diet (P = 0.28)		
Yes (n = 58)		No (n = 2)
0.2%		0.5%
Intramammary administration of antibiotics at the end of the previous lactation period (P = 0.95)		
Yes (n = 21)		No (n = 39)
0.2%		0.2%
Nutritional modifications performed during the last stage of pregnancy (P = 0.005)		
Yes (n = 28)		No (n = 32)
0.3%		0.1%
Administration of anthelmintics at the end of pregnancy		
Yes (n = 60)		No (n = 0)
0.5%		n/a

Table II.xiii. (Continued).

Administration of anti-clostridial vaccination at the end of pregnancy		
Yes (n = 60)		No (n = 0)
0.5%		n/a
Administration of anti-mastitis vaccination at the end of pregnancy (*P* = 0.43)		
Yes (n = 25)		No (n = 35)
0.2%		0.2%
Experience of the farmer (*P* = 0.074)		
0 – 10 years (n = 13)	11 – 20 years (n = 30)	> 20 years (n = 17)
0.3%	0.1%	0.3%
Presence of working staff in the farm (*P* = 0.72)		
Yes (n = 39)		No (n = 21)
0.2%		0.2%

Details of the results of the multivariable analyses are in Table II.xiv.

Table II.xiv. Results of multivariable analysis for associations with death during the peri-parturient period [1] during a two-year study in 60 sheep flocks in Greece.

Variable	Proportion	Odds ratio[2] (95% confidence intervals)	*P* value
Occurrence of death during the before lambing component of the peri-parturient period			
Season of the year during which the lambing period took place			0.002
Autumn (n = 36)	0.3% (23 / 7513)	reference	
Winter (n = 5)	1.3% (18 / 1389)[3]	3.088 (1.560–6.111)	0.001
Spring (n = 0)	n/a	n/a	n/a
Summer (n = 19)	0.7% (33 / 4589)	2.359 (1.383–4.022)	0.002

Table II.xiv. (Continued).

Variable	Proportion	Odds ratio[2] (95% confidence intervals)	*P* value
Occurrence of death during the after lambing component of the peri-parturient period			
Number of animals in the flock			0.024
≤ 200 ewes (n = 38)	0.4% (18 / 5092)	3.990 (1.174–13.555)	0.027
200–500 ewes (n = 17)	0.2% (8 / 4944)	1.829 (0.483–6.876)	0.375
> 500 ewes (n = 5)	0.1% (3 / 3377)	reference	

1. The figures refer only to cases of death that occurred within the 18-day long peri-parturient period.
2. Odds ratio calculated against the lowest prevalence associations of the variables.
3. n/m: number of ewes that died the respective component of the peri-parturient period / number of ewes at risk at the start of the respective component of the peri-parturient period (numbers averaged per flock from results of the two years of the study).

Occurrence of death during each of the three components of the peri-parturient period in each of the two years of the study

When the analyses for occurrence of death during each of the three components of the peri-parturient period were repeated with the results of each year of the study considered separately, the findings were generally similar (Table II.xv).

Table II.xv. Summary of results of analysis for associations with death during the peri-parturient period, during a two-year study in 60 sheep farms in Greece, with the results of each year of the study considered separately.

Occurrence of death: during the before lambing component of the peri-parturient period	
1st year of the study	
Significant variables in the univariable analysis	*p*
Management system applied in the flock	< 0.0001
Season of the year during which the lambing period took place	< 0.0001
Experience of the farmer	< 0.0001
Significant variables in the multivariable analysis	*p*
Management system applied in the flock	0.018
Season of the year during which the lambing period took place	0.046
Experience of the farmer	0.025

Table II.xv. (Continued).

Occurrence of death: during the before lambing component of the peri-parturient period	
2nd year of the study	
Significant variables in the univariable analysis	*p*
Season of the year during which the lambing period took place	< 0.0001
Experience of the farmer	0.022
Most significant variable in the multivariable analysis	*p*
Season of the year during which the lambing period took place	0.09
Occurrence of death: during the at lambing component of the peri-parturient period	
1st year of the study	
Significant variables in the univariable analysis	*p*
n/a	
Most significant variable in the multivariable analysis	*p*
Presence of working staff in the farm	0.28
2nd year of the study	
Significant variables in the univariable analysis	*p*
Season of the year during which the lambing period took place	0.001
Breed of ewes	0.022
Control of reproduction	0.0005
Presence of working staff in the farm	0.002
Significant variable in the multivariable analysis	*p*
Presence of working staff in the farm	0.030
Occurrence of death: during the after lambing component of the peri-parturient period	
1st year of the study	
Significant variables in the univariable analysis	*p*
Number of animals in the flock	0.004
Breed of ewes	< 0.0001
Control of reproduction	0.002
Nutritional modifications performed during the last stage of pregnancy	0.002
Experience of the farmer	0.022

Table II.xv. (Continued).

Occurrence of death: during the after lambing component of the peri-parturient period	
1st year of the study	
Most significant variable in the multivariable analysis	*p*
Control of reproduction	0.06
Occurrence of death: during the after lambing component of the peri-parturient period	
2nd year of the study	
Significant variables in the univariable analysis	*p*
Number of animals in the flock	0.046
Nutritional modifications performed during the last stage of pregnancy	0.031
Significant variable in the multivariable analysis	*p*
Nutritional modifications performed during the last stage of pregnancy	0.046

GENERAL DISCUSSION

Introduction

This study explored in detail, for the first time internationally, the mortality of ewes during the peri-parturient period. Hindson and Winter (1990) and Mavrogianni and Brozos (2008) were the first to describe the problem; however, those reports were based on the clinical experience of the authors rather than on data from organised clinical research work.

In contrast, the present paper presents an extensive study performed in 60 flocks of dairy sheep over two successive years. Peri-parturient mortality has a low incidence; therefore, extent of the study over two years has provided helpful information on the sporadic occurrence of the problem from year to year and also allowed a more thorough exploration of possible risk factors.

Incidence risk of cases of death during the peri-parturient period in the sheep flocks

First, the findings confirmed that case of death during the peri-parturient period accounted for most cases of death of adult ewes in the sheep flocks: almost 60% of the cases of death in the study. Further, the findings indicated that the problem had a sporadic occurrence. The incidence risk was < 1.0% and the problem was recorded in only a few flocks during both years.

The low incidence of peri-parturient mortality should not undermine its importance for sheep flocks. Cases of death during the peri-parturient period have a significant impact on the flock. To reach the peri-parturient period, the ewes were subjected to an intensive preparation, while high input from farmers was required as well. This input included the use of infrastructure, the feeding and the intensive labour provision, as well as the extended veterinary care for pregnancy diagnosis, and administration of vaccines and pharmaceuticals (Fthenakis et al. 2012); hence, if a ewe died there would be no return on investment. In such cases, the capital investment (i.e., ewes) perishes along with all the work in anticipation for the lambing; these expenses can often add up to a significant proportion of the value of a ewe. The importance is similar in dairy- and meat-production systems. In a recent study of ewe wastage in meat production systems in New Zealand, a much higher removal rate from the flocks from mating to lambing were noted (Flay et al. 2021) compared to this study. This can be explained if one considers that in dairy systems, ewes would be milked until 30 to 45 days before the expected lambing (i.e., farmers would provide care and infrequently cull animals until after the end of the lactation period).

In the present study, no records were available regarding the ewes that had been culled. Inevitably, this possibly led to a slight overestimate of the number of ewes and ewe-days at risk, so the estimated peri-parturient mortality rates are likely to be slightly below their true values. Because of the husbandry systems used in these dairy flocks, very few animals were culled. Our estimates were indeed very close to the true values, because (a) the pregnancy rate is normally > 98% due to the extended breeding season, (b) ewes were milked until 30 to 40 days before the expected start of the lambing period and (c) the ewes were often kept until the end of the reproductive year when there is a market for their meat.

Time of death in relation to the components of the peri-parturient period

Among cases of death during the peri-parturient period, the highest incidence rate was seen before lambing and was aligned with the clinical conditions associated with the deaths (i.e., pregnancy toxaemia, which is the most important metabolic disorder of pregnant ewes).

The lack of any statistical difference with the incidence rate at lambing was in partial accord with the previous suggestions of Hindson and Winter (1990) and Mavrogianni and Brozos (2008), who previously had indicated that most cases of death during the peri-parturient period occurred at lambing time.

Pathological conditions associated with cases of death during the peri-parturient period

Pregnancy toxaemia, the clinical condition most commonly associated with death, was the most important metabolic disease of sheep and occurred frequently in all flocks (up to 10% in undernourished flocks (Sargison 2008). The disease can be fatal, especially if veterinary intervention is delayed. Another clinical condition often associated with the death of affected ewes was ruminal acidosis, which can be acute and result to death over the course of hours (Dijkstra et al. 2012, Hernández et al. 2014). These two conditions, cumulatively, accounted for over 50% of all cases of death during the peri-parturient period. The last stage of gestation is particularly demanding metabolically; hence, the energy requirements of pregnant ewes increase, as the end of pregnancy approaches. It is thus recommended that the nutritional regime of pregnant ewes be progressively

modified, with energy provisions to accommodate the increased requirements of the ewes (Fthenakis et al. 2012). Farmers may not always perform the necessary changes in the feeding regime in a timely manner, which can lead to pregnancy toxaemia, depending on various factors (Barbagianni et al. 2015a). As farmers see the problem developing, they would realise the necessity to increased energy and may attempt to rectify it by providing increased amounts of cereals, which can then lead to acidosis (in 6 of 7 flocks with cases of death associated with ruminal acidosis, deaths associated with pregnancy toxaemia were also recorded). The acute nature of these two situations is shown by the high mean number of ewes that died in each flock, in which these disorders predominated.

In winter, due to the low temperatures and increased precipitation, pregnant ewes have increased metabolic requirements (Symonds et al. 1988). These can further predispose them to pregnancy toxaemia. Indeed, in a recent report from Australia, increased ewe mortality was reported after a season of high precipitation (Watt et al. 2021). Moreover, other situations occurring during the winter may also play a role; for example, adverse weather conditions may hinder veterinary access to farms. All these contributed to the emergence of the association of winter with a death before lambing.

Dystocia was the only pathological condition associated with death at the time of lambing. The lack of farm staff was found to contribute, to some extent, to this; this was reasonable, as many lambings might occur during the night, and personnel assistance is crucial for dealing with such cases. Furthermore, most such cases occurred in primigravidae ewes, in which foeto–maternal disproportion occurs more frequently (Arthur and Bee 1996a); in such cases, the genital tract may not be fully developed, especially if the ewe-lambs were mated too young (Hindson and Winter 2007).

Finally, post-partum genital disorders and clinical mastitis were the pathological conditions associated with death of ewes after lambing. Many of the genital disorders might have been the sequelae of dystocia that were dealt with inappropriately (e.g., uterine rupture (Hindson and Winter 2007)). Obstetrical manipulations without maintaining aseptic conditions can lead to metritis (Bolinder et al. 1988), and uterine and rectal prolapse are often the consequences of repeated straining by parturient ewes (Arthur and Bee 1996b). It is also noteworthy that pregnancy toxaemia can often lead to post-partum problems of the genital tract (Barbagianni et al. 2015c, Ioannidi et al. 2020) and has been associated with the development of clinical mastitis during the first week post-partum (Barbagianni et al. 2015b). Hence, one can postulate that, in some flocks, ewes with pregnancy toxaemia that had received successful veterinary attendance, later developed problems post-partum, as described in previous studies (Barbagianni et al. 2015c, Ioannidi et al. 2020). It is also possible, given the immunocompromise present during that period, that the affected ewes were

unable to counteract a mammary infection, and thus developed acute clinical mastitis and consequently died (Kandefer-Szerszen et al. 1992, Lacetera et al. 2005). Moreover, in animals with increased concentrations of β-hydroxybutyrate (which occurs in pregnancy toxaemia), Galvão et al. (2010) found reduced blood neutrophil numbers, an issue that can clearly predispose a ewe to mastitis, given the importance of neutrophils in the defence mechanisms of the mammary gland (Katsafadou et al. 2019).

Factors associated with occurrence of death during the peri-parturient period

It was interesting to note that while differing predictors were identified for death before or after lambing, another one, the average lambing rate at flock level, was found to be significant for the occurrence of death independent of the stage of lambing. A higher average lambing rate at flock level (over 1.5) was found to be associated with the increased risk of death during the peri-parturient period. It is possible that the increased lambing rate could connect all the issues mentioned above. The large number of foetuses predisposes ewes to pregnancy toxaemia (Sargison 2008) and increases the risk of dystocia (Bolinder et al. 1988); furthermore, an increased litter has been positively associated with the development of mastitis (Waage and Vatn 2008, Vasileiou et al. 2019) and metritis (Bell and Roberts 2007, Dubuc et al. 2010).

Conclusions

The conclusions from the results of the present thesis are summarised herebelow.

(a) Death during the peri-parturient period was found to occur sporadically and most cases occurred 10 days prior to lambing.

(b) Pregnancy toxaemia was the pathological condition most frequently associated with peri-parturient mortality.

(c) Factors found to have a significant association with occurrence of peri-parturient mortality included the average lambing rate at flock level (increased risk with average lambing rate > 1.5), the season of the year during which the lambing period took place (highest risk during the winter), and the number of animals in the flock (highest risk in the flock with ≤ 200 ewes).

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