



## Forensic Matters

## Forensic investigations of suspected livestock depredation by vultures: scientific tools for compensation programmes

Agustín Rebollada-Merino <sup>a,\*</sup>, Alberto Gómez-Buendía <sup>b,c</sup>, Lucas Domínguez <sup>b,c</sup>, Antonio Rodríguez-Bertos <sup>b,d</sup>

<sup>a</sup> Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, 240 Farrier Rd, Ithaca, NY 14850, USA

<sup>b</sup> VISAVET Health Surveillance Centre, Complutense University of Madrid, Madrid 28040, Spain

<sup>c</sup> Department of Animal Health, Faculty of Veterinary Medicine, Complutense University of Madrid, Madrid 28040, Spain

<sup>d</sup> Department of Internal Medicine and Animal Surgery, Faculty of Veterinary Medicine, Complutense University of Madrid, Madrid 28040, Spain

## ARTICLE INFO

## Article history:

Received 12 July 2023

Accepted 14 January 2024

## Keywords:

forensic pathology  
histopathology  
human–wildlife conflicts  
livestock depredation  
vulture

## ABSTRACT

Human–wildlife conflicts may have devastating consequences for fauna due to targeting by humans of wildlife populations suspected to have predated livestock. Suspicion of depredation of extensively raised livestock by vultures in Europe has triggered public administration-led forensic investigations intended to distinguish between predation and scavenging in order to compensate farmers for attacks on their livestock. In this study, gross and histological analyses were carried out on suspected cases of domestic animal depredation by griffon vultures (*Gyps fulvus*) over a 1-year period. Fifty-eight animals were affected ( $n = 41$ , suspected depredation), including domestic cattle (75.9%), sheep (22.4%) and a goat (1.7%). All the adults affected were female and most cases of suspected depredation occurred during the peripartum period (56%). Histological investigations distinguished between post-mortem (84% of diagnostic samples) and ante-mortem (16% of diagnostic samples) cases, and gross examinations revealed significant differences ( $P \leq 0.002$ ) between ante-mortem, post-mortem and non-diagnostic samples. This study highlights the need to optimize sampling protocols to increase the success of forensic studies. The forensic investigations presented here may be applied to resolve human–wildlife conflicts involving not only vultures but other endangered carnivores and contribute to human–wildlife coexistence in rural areas by protecting the interests of both the livestock sector and endangered species.

© 2024 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

## 1. Introduction

Vultures are scavenger birds that are divided into two families: *Accipitridae* (Old World vultures), which are located in Europe, Africa and Asia, and *Cathartidae* (New World vultures), which are located in America [1]. Of the 23 extant vulture species, nine are critically endangered [2,3]. Global vulture populations started to decline in the mid-19th century, mainly due to anthropogenic causes [4], but have stabilized during the 21st century. Today vultures are still persecuted due to ignorance, superstition and human retaliation in response to alleged predation of domestic animals [2–4]. As obligate scavengers, vultures subsist entirely on muscle, viscera and bones from carcasses, as opposed to facultative scavengers, which gain most of their food through predation. Vultures

have overcome the spatial and temporal challenges of feeding on carrion, an ephemeral and generally patchily distributed resource [5–7]. For vultures, food availability depends on live prey abundance and mortality. Changes in vulture abundance are believed to be related to disease transmission patterns [6,7].

In 2002, the European Union (EU) implemented health policies to prevent outbreaks of bovine spongiform encephalopathy (EC1774/2002), dictating carcass destruction and indirectly threatening the conservation of scavenger birds [8–10]. This was the subject of a legal dilemma: destroying all carcasses (the main food source for scavengers) versus strictly protecting scavenger species. Subsequently, a consensus among scientists, conservationists and managers considered the natural feeding patterns of scavengers, allowing farmers to leave livestock carcasses in scavenger feeding zones (CE 322/2003, CE 830/2005, CE 142/2011) [10]. Spain designated scavenger feeding areas and added additional criteria through national and regional laws. The lack of consistent scientific criteria at the national level has resulted in a shortage of

\* Corresponding author.

E-mail address: [amr499@cornell.edu](mailto:amr499@cornell.edu) (A. Rebollada-Merino).

scavenger food in some areas, which may have altered the foraging behaviours of scavengers, with detrimental socioeconomic and ecological consequences [9,10].

The most relevant behavioural change is livestock predation by griffon vultures (*Gyps fulvus*) in Spain and France [9]. As obligate scavengers, vulture predation is regarded as an altered behaviour. The cause is attributed to an 'increase in the vulture population due to the success of conservation campaigns [...] and decreased availability of carrion' [9]. The devastating consequences of this conflict for the European vulture population are discontent and action against these birds by some affected farmers [4,9]. Misinformation disseminated through social media has magnified the actual incidence of these depredations [4]. The growing suspicion of livestock depredation by vultures in Spain has triggered the commissioning of forensic investigations by the Spanish public authorities with the aim of reliably distinguishing between scavenging and predation by vultures on livestock in order to (1) compensate farmers for the economic losses in cases due to confirmed depredation and (2) prevent human attacks on wildlife populations. We anticipate that the results presented in this study will contribute to the resolution of conflicts concerning suspected predation of livestock and protect the interests of farmers and endangered wildlife.

## 2. Materials and methods

### 2.1. Cases included and study area

Suspected cases of vulture predation on livestock (extensively raised cattle, sheep and goats) over a 1-year period (June 2020 to May 2021) in an autonomous region of Spain were included. The studied region has a Mediterranean climate, with annual mean temperatures ranging from 4°C in winter to 26°C in summer. Precipitation is 400–600 mm per year and humidity ranges from 37% to 50%. The mean altitude is 696 metres.

### 2.2. Epidemiological and sampling data

Time of year, animal species, number of individuals affected, age, sex and occurrence of a recent partum (peripartum) in adult animals were recorded in each case. The interval between farmer suspicion of depredation by vultures and their notification to environmental law enforcement agents was recorded. Sampling by environmental law enforcement agents occurred within 24 h after notification.

### 2.3. Gross pathology study

The two veterinary pathologists involved in this study trained environmental law enforcement agents to follow the same protocol for the photographic reporting of carcasses and the collection of tissue samples for histological studies and ancillary tests. Photographs were taken of the environment.

The cadaver was photographed from a minimum of four different angles, to include the externally visible mucosae (oral, nasal, conjunctival, anal and genital), suspected external lesions and internal organs if present. Veterinary pathologists analysed the gross images recording the anatomical locations affected (head, thorax, abdominal–pelvic area and limbs). The samples collected for histological studies comprised intact skin, suspected external lesions (including skin and appendages, subcutaneous tissue and associated glands, and skeletal muscle) and internal organs if available. When it was possible to refrigerate or freeze the samples, a swab was taken from areas of suspected lesions and liver and lung if present in the carcass.

### 2.4. Histological study

Tissue samples collected were fixed in formalin and subjected to standard histological processing. Slides were automatically stained with haematoxylin and eosin (HE). Histological examination classified samples as *ante mortem*, *post mortem* or undetermined. Cases classified as *ante mortem* had evidence of haemorrhage, necrosis or acute inflammation in the samples collected from grossly observed external lesions. Cases in which histological specimens of grossly suspicious lesions had no evidence of such changes were classified as *post mortem*. Undetermined cases were classified as such if autolysis and putrefaction precluded histological evaluation or if samples from grossly observed external lesions were not collected for histological examination.

### 2.5. Statistical analysis

The presence of lesions in samples between anatomical locations (head, abdomen and pelvis, thorax and limbs) was compared using a Kruskal–Wallis test followed by a Holm–Bonferroni post-hoc test. Differences between time intervals across groups (diagnostic and non-diagnostic [autolytic] samples) and subgroups (*ante mortem*, *post mortem* or undetermined samples) were determined using a one-way ANOVA test.

## 3. Results

### 3.1. Epidemiology

A total of 41 suspected depredations by vultures occurred, most of them in the autumn ( $n = 15$ , 36.6%; September,  $n = 0$ ; October,  $n = 5$ ; November,  $n = 10$ ) and winter ( $n = 15$ , 36.6%; December,  $n = 6$ ; January,  $n = 8$ ; February  $n = 1$ ), while a few occurred in the spring ( $n = 9$ , 22.0%; March,  $n = 3$ ; April,  $n = 1$ ; May,  $n = 5$ ) and summer ( $n = 2$ , 4.8%; June,  $n = 1$ ; July,  $n = 1$ ; August,  $n = 0$ ) (Supplementary Table 1).

Suspected depredation events involved from one to four animals, and altogether 58 animals were affected. Species suspected of being depredated by vultures included cattle ( $n = 44$ , 75.9%), sheep ( $n = 13$ , 22.4%) and a goat ( $n = 1$ , 1.7%) (Supplementary Table 1).

The cattle affected were mainly calves (33/44, 75.0%) but also some adults (11/44, 25.0%). The adults were all female; eight ranged from 3 to 13 years (mean: 6 years) and three were of unknown age. The calves affected were neonates (<1 day old, 12/33, 36.4%), 1 day old (2/33, 6.0%), 2 days old (2/33, 6.0%), 6 days old (1, 3.0%) or 7 days old (1, 3.0%) with the others (15/33, 45.6%) ranging from 13 to 82 days of age (mean: 30 days) (Supplementary Table 1).

The affected sheep were adults (7/13, 53.8%) and lambs (6/13, 46.2%). The adults were all female; three ranged from 3 to 4 years (mean: 4 years) and four were of unknown age. The lambs affected were neonates (<1 day old), 2 days old or 4 days old (2/6 each, 33.3%). The affected goat was a 2-day-old kid ( $n = 1$ ) (Supplementary Table 1).

The occurrence of a recent parturition (peripartum period; ie, less than 48 h after delivery) was recorded in 56% of suspected depredation events in all three species (23/41). Of these, one was a late-term pregnancy (1/23, 4.4%; case no. 41), one was a delivery associated with death of the cow (calf alive) (1/23, 4.4%; case no. 40), one was an abortion (1/23, 4.4%; case no. 4), one was a stillbirth (1/23, 4.4%; case no. 38), three were dystocias (3/23, 13.0%; cases no. 6, 33 and 39), six were post-natal deaths of the calf or lamb (6/23, 26.0%) and 10 were peripartum incidents not otherwise specified (10/23, 43.4%), of which seven involved the death of the dam and the offspring (cases no. 1, 5, 8, 9, 20, 24 and 35) and three involved

solely the offspring (cases no. 10, 19 and 34). In the other cases (18/41, 44%) it was not known if death had occurred during the peripartum period (not registered by environmental law enforcement agents) (Supplementary Table 1).

### 3.2. Gross findings

The distribution of affected anatomical areas in cattle varied between adults and calves. Adults ( $n = 11$ ) showed patterns of vulture feeding mainly in the abdominal/pelvic areas (11/11, 100%) affecting the perineum, ventral abdomen and internal organs (Fig. 1a and b). The head was also affected (5/11, 45.5%), involving mainly the eyes, tongue and oral mucosa. The thoracic area (2/11, 18.2%) and limbs (2/11, 18.2%) were less frequently affected and had variable degrees of skeletalization with occasional absence of thoracic organs. In calves ( $n = 33$ ) the abdominal/pelvic regions (29/33, 88%), head (28/33, 85%), limbs (25/33, 76%) and thorax (21/33, 64%) were affected. There was a higher frequency of skeletalization in calves compared with adults (Fig. 1c and d; Supplementary Table 1).

In adult sheep cases ( $n = 7$ ) the abdominal/pelvic regions were mainly affected (7/7, 100%), followed by the head (6/7, 86%), thoracic (5/7, 71%) and appendicular (3/7, 43%) areas. The head (6/6, 100%) was affected in all lambs ( $n = 6$ ), followed by the abdomen/pelvis (5/6, 83%), thorax (4/6, 67%) and limbs (4/6, 67%) (Fig. 1e and f; Supplementary Table 1). In the single goat kid in the study ( $n = 1$ ), both the abdominal/pelvic and head regions were affected (Supplementary Table 1).

### 3.3. Histological findings

Tissue samples for histological investigations were collected from all cases included in the study. Cutaneous and subcutaneous samples (comprising associated glands such as mammary glands or salivary glands) and muscle from affected regions were submitted in 39/41 cases (95%) (Supplementary Table 1). In three of these cases, various other organs were submitted together with skin samples and the following changes were seen: lymph node (follicular hyperplasia) in case no. 12 (calf); heart (moderate, chronic, lymphocytic and eosinophilic, perivascular, myocarditis and epicarditis with valvular haemorrhages) in case no. 14 (calf); and lung (desquamative alveolitis and alveolar emphysema), heart and bone marrow in case no. 17 (calf). In two cases (2/41, 5%; cases no. 22 and 24), internal organ samples (lung, spleen, abomasum) were collected but not skin samples. In case no. 22, the lung had lesions of chronic lymphoplasmacytic interstitial pneumonia, with meconium aspiration. The other organs of cases 22 and 24 did not have any relevant histological changes. These two cases were categorized as undetermined in the reports due to the impossibility of determining the ante-mortem or post-mortem condition of the affected skin regions. Ante-mortem lesions were identified in 4/39 cases (10%; cases no. 3, 38, 39 and 41) (Supplementary Table 1). Of the diagnostic cases (ie, those with non-autolytic skin samples), 4/25 (16.0%) were classified as *ante mortem*. In all cases, there were extensive haemorrhages in the skin and subcutaneous tissue characterized by diffuse separation of the dermal collagen fibres and expansion of the interstitium in the subcutaneous adipose tissue due to massive extravasation of erythrocytes (Fig. 2a). These haemorrhages also affected adnexal structures including hair follicles, sebaceous glands, sweat glands and salivary glands in perioral lesions of case no. 3 (kid). In case no. 39 (cow, dystocia), there were haemorrhages in both the acini and interstitium of the mammary gland (Fig. 2b), deposition of a disorganized fibrillar hyper-eosinophilic material (fibrin) and mild neutrophilic infiltration. In all cases the superficial skin regions had a multifocal to diffuse, partial (erosion) or complete (ulceration) loss of the epidermis (Fig. 2c).

Furthermore, in cases no. 38 (cow, stillbirth) and 41 (sheep, late-term pregnancy), in the skeletal muscle underlying affected regions of skin and subcutaneous tissue, fibres were multifocally swollen and hypereosinophilic, with loss of transverse striations of the sarcoplasm and karyorrhexis (Fig. 2d). The skeletal muscle interstitium was multifocally expanded by abundant extravasated erythrocytes (haemorrhage) and occasional scattered neutrophils and macrophages were observed.

Of the skin samples submitted, in 21/39 (54%) the changes seen were categorized as having occurred *post mortem* due to the lack of acute lesions or presence of non-severe, chronic, non-specific lesions (Supplementary Table 1). Of the diagnostic cases (ie, those with non-autolytic skin samples), 21/25 (84.0%) were classified as having occurred *post mortem*.

In 14/39 samples (36%), autolysis and putrefaction processes prevented appropriate histological assessment, and these were categorized as undetermined (Supplementary Table 1).

### 3.4. Relationship between gross and histological diagnoses

Cases classified as *ante mortem* commonly had involvement of the abdominal/pelvic areas (3/4, 75.0%) and head (3/4, 75.0%), followed by the limbs (2/4, 50.0%) and thorax (1/4, 25.0%). Post-mortem cases involved the abdominal/pelvic areas (20/21, 95.2%), head (15/21, 71.4%), thorax (13/21, 61.9%) and limbs (11/21, 52.4%). Cases in which histological examination revealed autolysis and putrefaction had a grossly affected abdomen/pelvis (14/14, 100%), followed by the head (11/14, 78.6%), limbs (11/14, 78.6%) and thorax (9/14, 64.3%) (Fig. 3, Supplementary Table 1). The presence of lesions between anatomical locations was significantly different ( $P \leq 0.002$ ). Post-hoc analysis revealed that these differences were based on the greater involvement of the abdominal and pelvic areas compared with the other anatomical locations. The relationship between gross and histological diagnoses could not be determined for the two cases for which skin samples were not available.

### 3.5. Suspected depredation–notification interval analysis

The suspected depredation–notification intervals recorded for diagnostic (ante-mortem and post-mortem) and autolytic samples were significantly different ( $P = 0.03$ ). They ranged from 0 to 240 h (mean: 24 h; median: 14 h) and, as expected, samples that had undergone autolysis and putrefaction had a higher depredation–notification interval (mean: 46 h; median: 21 h) than those in which a reliable histological analysis was made (mean: 12.2 h; median: 8 h) (Supplementary Table 1). On the other hand, no statistically significant differences were observed across subgroups, although samples classified as having occurred *ante mortem* had reduced depredation–notification intervals (mean: 6 h; median: 5 h) compared with post-mortem (mean: 13 h; median: 9 h) and autolytic samples (mean: 46 h; median: 21 h) (Fig. 4).

## 4. Discussion

The improvement of human and wildlife coexistence in rural areas should equally consider livestock sector interests and endangered species. In the EU, coexistence is achieved through conflict prevention and compensation programmes that aim to mitigate the economic losses of farmers [11]. The first steps to a successful compensation programme are, *inter alia*, quick and accurate verification of the damage and prompt and fair payments (retrospectively) [12]. Thus, studies focusing on the scientific aspects of human and wildlife conflicts are the basis of successful compensation programmes. These investigations are essential for veterinarians to diagnose those cases accurately (verification of the

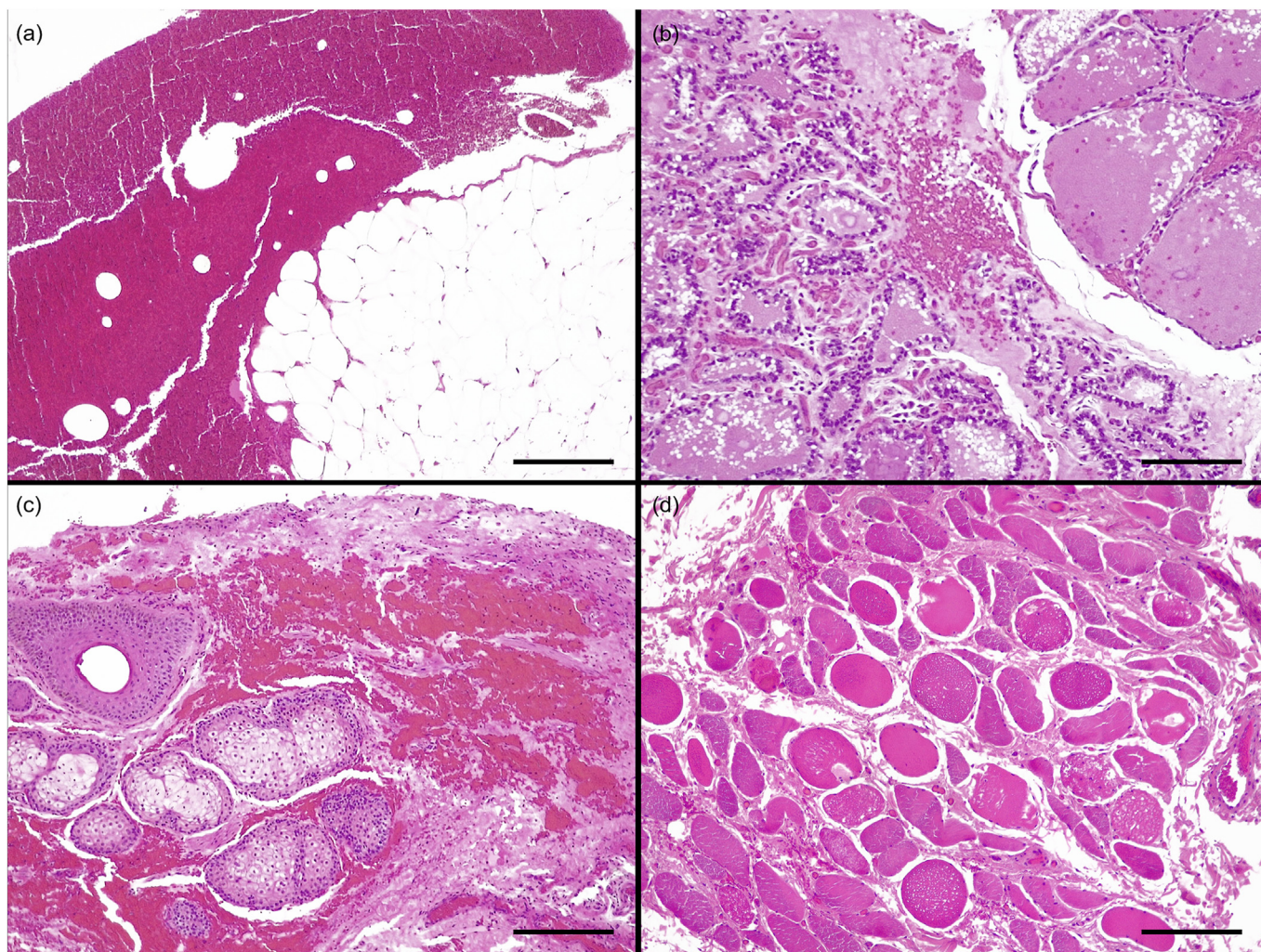


**Fig. 1.** Suspected vulture attacks on livestock. (a) Case 39. *Ante mortem*. Cow and calf death due to dystocia. Vulvar haemorrhages and partial consumption of calf. (b) Case 38. *Ante mortem*. Vulva of cow has multifocal areas of dermal and mucosal ulceration, and haemorrhage. (c) Case 37. *Post mortem*. One-day-old calf with partial scavenging of abdominal and pelvic areas. (d) Case 32. *Post mortem*. Two-day-old calf with partial scavenging of facial region including ears, eyes, rostral nose and mouth. (e) Case 20. Autolysis and putrefaction. Scavenged sheep almost totally skeletonized. (f) Case 24. Undetermined. Lamb with absence of rostral tongue.

damage), thus providing appropriate assistance to the environmental law enforcement agents and public administrations.

From a veterinary forensic perspective, the scavenging pattern of vultures is unexplored. Indeed, available information has been

extrapolated from a few regional studies using pig or human carcasses as models to study vulture feeding on human remains [13–16]. Vultures have a specialized beak consisting of a cutting edge and a curved tip that tear fibres from bones efficiently [17].



**Fig. 2.** Ante-mortem vulture attacks on livestock. (a) Case 41. Sheep. Extensive haemorrhages in subcutaneous tissue. (b) Case 39. Cow. Multifocal haemorrhages in mammary gland. (c) Case 3. Two-day-old kid. Epidermal ulceration and multifocal to coalescing haemorrhages in dermis and cutaneous adnexa. (d) Case 38. Cow. Muscle necrosis and haemorrhage in perineal tissue. All HE. Bar, 200  $\mu$ m.

Vulture feeding begins in easily accessible body areas (mouth, eyes and anus) and continues through the abdomen (skin, muscles, viscera, tendons and bones) [17]. In this study, the feeding pattern observed was compatible in all cases but there were differences between the carcasses of young and adult animals. The abdominal and pelvic areas were consumed to a greater degree than other anatomical regions in adult carcasses, suggesting that vulture feeding begins in the perineal area. In contrast, in calves and lambs there was a more heterogeneous feeding pattern affecting not only the abdominal and pelvic cavities but also the head and limbs in >75% of cases. This pattern may be explained by the reduced size of calf and lamb carcasses, which decreases the time needed to consume all the internal tissues. These results facilitate differentiation of the feeding patterns of vultures from those of other wildlife that may potentially be involved in livestock deaths. With regard to the relationship between the gross pattern and histological diagnosis, a higher proportion of affected anatomical areas was observed in the undetermined cases compared with the ante-mortem and post-mortem cases, suggesting a delay in sampling and a longer time for carcass consumption by the vultures.

Necropsy is invaluable for determining the cause and manner of death and provides relevant information to implement appropriate sanitary management of herds and flocks [18]. In this investigation,

sampling was performed in the field by environmental law enforcement agents as they are the professionals in charge of the examination of the scene in Spain. Furthermore, the remote location of the carcasses and limited economic resources for these investigations made submission of these cases for necropsy unfeasible. Accepting these limitations, whenever possible, performing the most complete necropsy possible in suspected cases of livestock depredation by vultures is recommended. Post-mortem examination, including photographic documentation and sampling, should be performed by veterinarians. One of the major limitations of field diagnosis in these cases is rapid carcass consumption by scavengers. For this reason, tissue sampling for further histological interpretation by veterinary forensic pathologists should include several representative samples of skin from suspected lesions and non-affected areas, as well as internal organs if available. Sampling for eventual bacteriological and molecular studies is highly recommended [18] but is hampered by sample preservation if the event occurs in remote locations (data not shown). However, integration of these diagnostic techniques can serve to exclude serious debilitating diseases or life-threatening conditions in livestock suspected to have been depredated by vultures.

Somatic death is the cessation of cardiac and respiratory function that precedes cellular death [19]. After death, there is

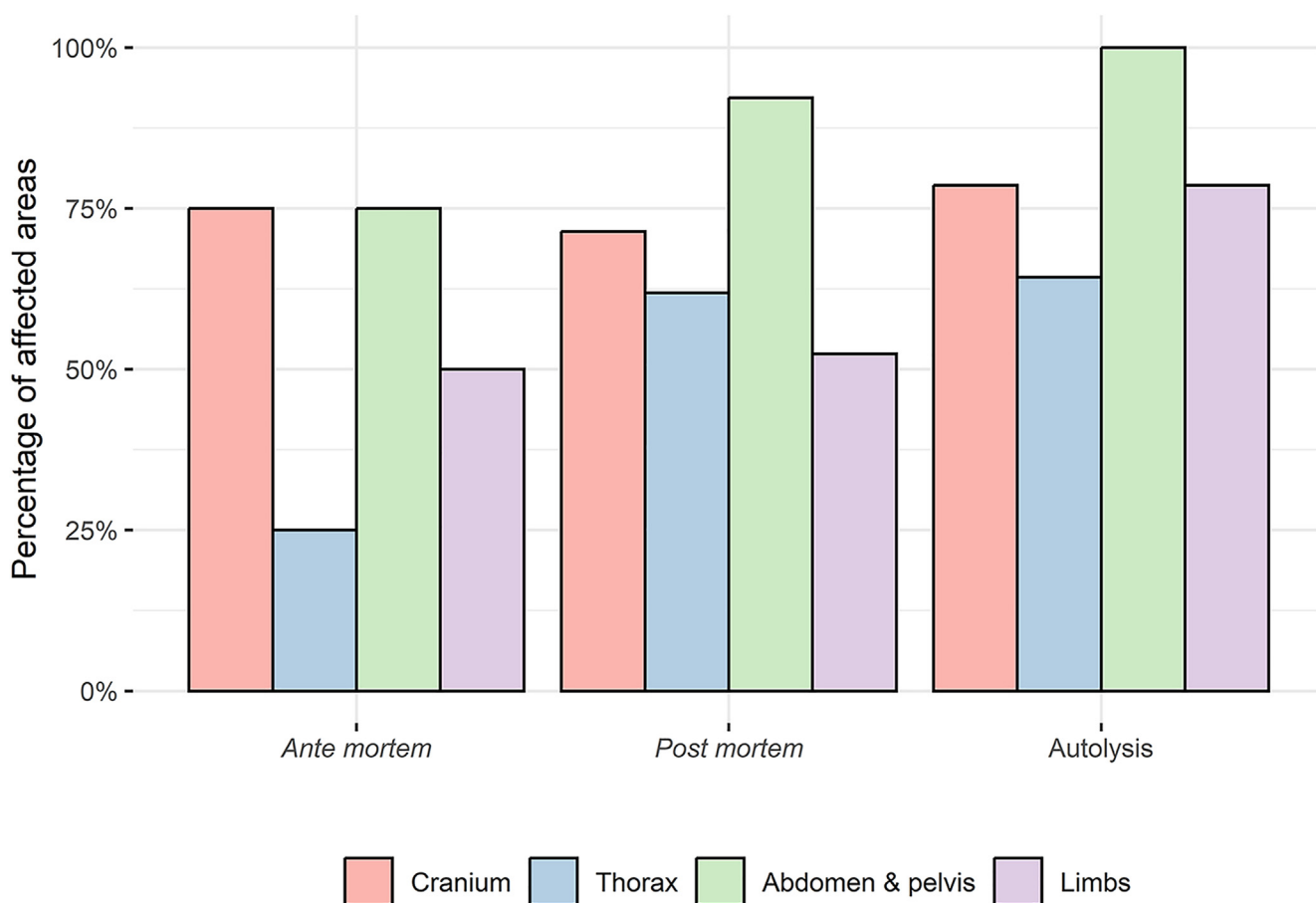


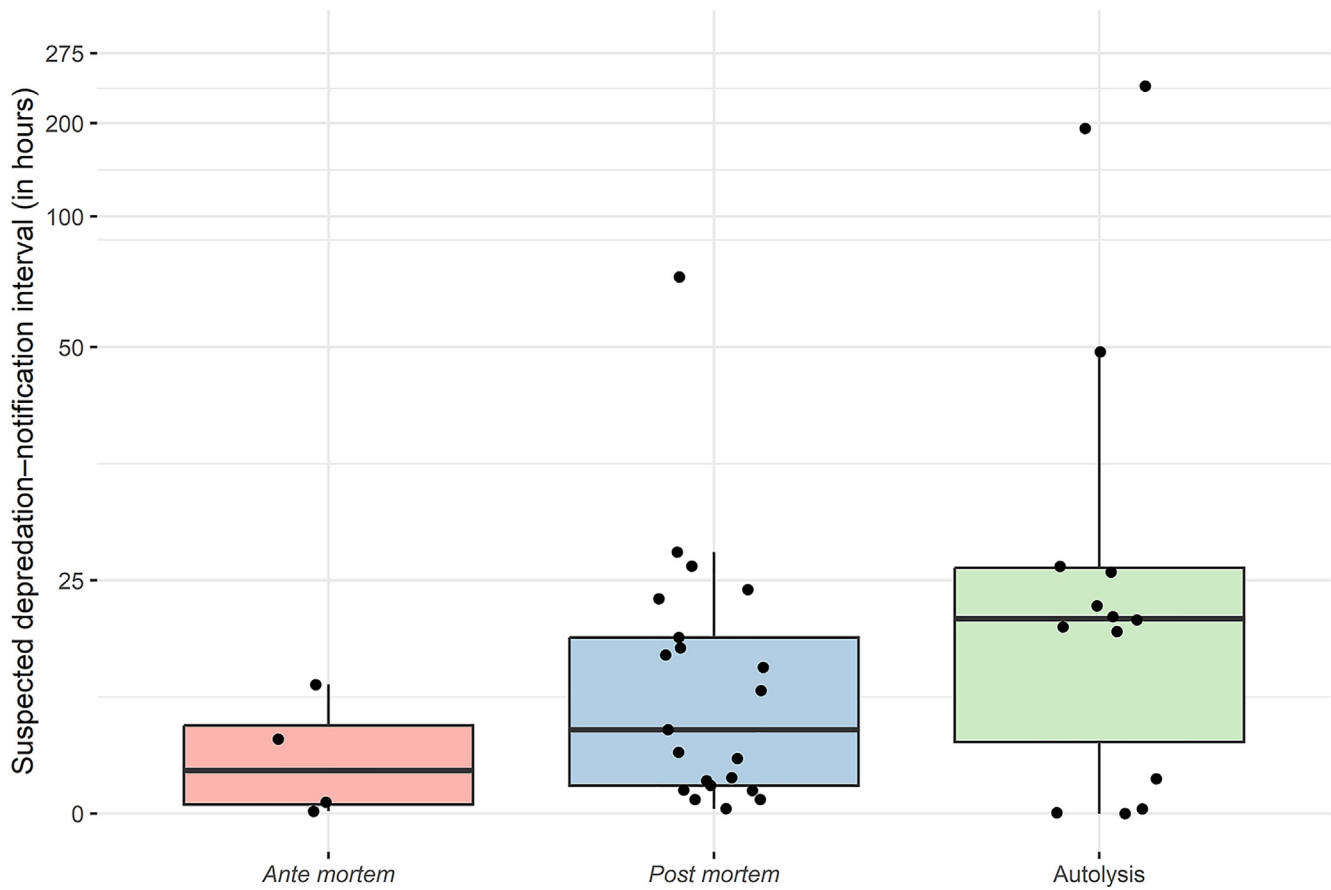
Fig. 3. Percentage of anatomical regions affected in ante-mortem, post-mortem and non-diagnostic subgroups.

termination of haemodynamic processes, systemic inflammatory response and tissue repair. From a forensic perspective, histology is the gold standard technique for excluding or confirming vital reactions because ante-mortem insults induce tissue response by means of haemodynamic and/or inflammatory changes [20]. For field forensic investigations, histology has many logistical advantages. The main one is that formalin-fixed samples do not require special temperature conditions until submission, thus preserving tissue degradation, which is useful if sampling takes place in remote geographical areas [18]. Histological processing is also inexpensive compared with molecular techniques. However, histological interpretation requires trained and experienced veterinary pathologists [20]. Molecular-based assays based on predator salivary deoxyribonucleic acid (DNA) identification in carcasses have been used in the context of livestock depredation by wolves or dogs [21,22]. However, for forensic investigations involving scavengers, these methods do not distinguish between predation and scavenging, as scavenger DNA would be expected to be found in carcasses because of their normal feeding behaviour [21].

Some authors have demanded studies on the reliability, frequency and context of suspected vulture predation on livestock [4]. In our study, the ante-mortem nature of lesions was demonstrated by histology in 16% of diagnostic cases. Most diagnostic samples were determined to represent post-mortem cases, as reported by others [23,24]. A predatory role of American black vultures (*Coragyps atratus*) on domestic cattle, pig and sheep neonates has been reported [25–29] but those studies lacked histological confirmation. Such vulture behaviour was attributed to an attempt to obtain food

from the placenta during delivery with no intention of harming the neonate, but fatal injuries in a percentage of cases have been described [26,27]. In our study, all four cases classified histologically as *ante mortem* involved the peripartum period, suggesting that these events occurred in animals that had problems during parturition. Occasional predation of young and weak individuals by American black vultures in North America or white-headed vultures (*Trigonoceps occipitalis*) in South Africa has been described but the association with the peripartum period or poor sanitary conditions has not been addressed [30–32]. Our results confirm the suspicion of livestock depredation by vultures in the Iberian Peninsula [9] and support the need to develop or maintain compensation programmes in affected areas to prevent conflicts between humans and wildlife.

The preliminary results presented here assessed the incidence of livestock depredation by vultures in an autonomous region in central Spain over 1 year. Future broader studies employing this methodology should be performed in other Spanish regions as well as other countries to obtain a broader view of the magnitude of these events. The proportion of cases classified as *ante mortem* in our investigation may be an underestimate as evaluation of more than a third of the samples was hindered by autolysis and putrefaction processes. The suspected depredation–notification interval was significantly higher for autolytic samples compared with diagnostic samples (classified as having occurred *ante mortem* or *post mortem*). This factor is exclusively dependent on the suspected depredation–notification interval as communicated by farmers. Delay in notification not only promotes autolysis and putrefaction in uncontrolled environmental conditions but also favours skeletal



**Fig. 4.** Differences between subgroups according to the interval between attack and notification of incident. Boxes indicate interquartile range, horizontal bars within boxes indicate median values, and top and bottom of boxes indicate 75th and 25th percentiles, respectively. Whiskers denote 97.5th and 2.5th percentiles. Dots represent individual cases.

reduction of the carcass and, therefore, absence of available samples, since vultures are able to skeletonize a carcass within minutes *post mortem* [33]. Therefore, farmers must be made aware of the importance of promptly notifying these alleged depredations and, if possible, preserving the scene (covering the carcass with plastic prevents scavenging by vultures) because success of the subsequent investigation is largely dependent on prompt notification.

Cattle were involved in more suspected depredations than small ruminants. However, as the economic value of cattle is higher than small ruminants, overreporting for high value species is to be expected. Similarly, all the adult animals here were female and of high value due to their role in reproduction. More than a half of the suspected depredations occurred during the peripartum period. The peripartum period is known to be life-threatening in both domestic ruminant females and neonates [34]. In adult ruminants, several metabolic (pregnancy toxemia, ruminal acidosis, hypocalcaemia) and reproductive (eg, uterine prolapse, rectal prolapse, metritis, dystocia, mastitis) problems can lead to death [34]. Predation by wild animals during the peripartum period has been described among the causes of periparturient deaths in sheep [34] as found in our study.

In this investigation, suspected depredations involved a significant number of calves, lambs and goat kids. In-utero infections are major causes of full-term losses in domestic ruminants and several infectious agents can be involved in abortions, stillbirths and neonatal mortality [35–40]. We had intended to investigate the role of abortive agents in this study but could not do so due to the following limitations: (1) the zoonotic potential of some abortive agents would have constituted a health risk to the environmental

law enforcement agents (who have limited training in biosafety and handling of potentially dangerous biological samples); (2) the absence of target tissues for sampling due to carcass consumption by scavengers [38]; and (3) field research in remote locations hinders the preservation of biological samples for microbiological analysis. Moreover, detection of abortive agents is challenging and negative results do not exclude infection because autolysis and putrefaction, contamination and inappropriate tissue sampling techniques may hinder peripartum mortality investigation [38]. Our data also suggest the need to emphasize reproductive infectious disease surveillance in extensively raised livestock in the studied area in order to establish the sanitary status. A low sanitary status of flocks or herds may interfere with the results of forensic investigations as increased livestock mortality may be misinterpreted as depredations by wildlife.

Perinatal mortality (death within 48 h *post partum*) was a significant finding in our study, as reported in beef calves [37,40–44]. This is considered a welfare indicator aside from the negative economic consequences for farmers [38]. Important causes of death in neonatal calves and young animals are diarrhoea, pneumonia, congenital malformations and adverse environmental factors [45–49]. In this investigation, most suspected depredations occurred in the coldest months (September–February) and it is known that peripartum mortality in cattle and sheep is increased in the winter months [34,42]. Furthermore, as calving at pasture is a risk factor for perinatal mortality in extensively raised systems, pre- and post-calving shelter is recommended to help improve supervision during and immediately after delivery [38,43,50,51]. Dystocia

is a leading cause of death in neonatal calves, has a complex aetiology [41,44] and not only may cause the death of calves during delivery but can lead to increased neonatal morbidity due to interference with colostrum intake and consequent acquisition of passive immunity [40,50]. In this investigation, dystocia and twin calving were each recorded in 23.1% of cases. Twin calving is associated with a higher risk of life-threatening reproductive problems (dystocia, retained placenta, metritis, abortion, stillbirth and neonatal calf mortality) and/or metabolic problems in ruminants [52].

## 5. Conclusion

Forensic field investigations of suspected domestic animal depredation by wildlife are challenging and require a scrupulous methodology and a multidisciplinary veterinary approach. Forensic cases of suspected livestock depredation by vultures should ideally include necropsy, histology and sampling for ancillary testing. Intravital diagnosis of these cases should be complemented by diagnosis of the final cause of death, given the involvement of the peripartum period and neonates in many cases.

## Funding

There was no specific funding for this work.

## Acknowledgments

The authors thank G. Torre (VISAVET Health Surveillance Centre, Complutense University of Madrid) for her technical support. We are indebted to all the environmental law enforcement agents (Agentes de Medio Ambiente) for their field work.

## Declaration of competing interests

The authors declared no conflicts of interest in relation to the research, authorship or publication of this article.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jcpa.2024.01.006>.

## References

- [1] Buechley ER, Sekercioglu CH. Vultures. *Curr Biol* 2016;26(13):R560–1.
- [2] Koenig R. Vulture research soars as the scavengers' numbers decline. *Science* 2006;312(5780):1591–2.
- [3] Ogada D, Keasing F, Virani MZ. Dropping dead: causes and consequences of vulture population declines worldwide. *Ann N Y Acad Sci* 2011;1249(1):57–71.
- [4] Lambertucci SA, Margalida A, Speziale KL, Amar A, Ballejo F, Bildstein KL, et al. Presumed killers? Vultures, stakeholders, misperceptions, and fake news. *Conserv Sci Pract* 2021;3(6):e415.
- [5] Houston DC, Cooper J. The digestive tract of the whiteback griffon vulture and its role in disease transmission among wild ungulates. *J Wildlife Dis* 1975;11(3):306–13.
- [6] Kendall CJ, Virani MZ, Hopcraft JGC, Bildstein KL, Rubenstein DI. African vultures don't follow migratory herds: scavenger habitat use is not mediated by prey abundance. *PLoS One* 2014;9(1):e83470.
- [7] Ogada D, Torchin ME, Kinnaird MF, Ezenwa VO. Effects of vulture declines on facultative scavengers and potential implications for mammalian disease transmission. *Conserv Biol* 2012;26(3):453–60.
- [8] García-Alfonso M, Van Overveld T, Gangoso L, Serrano DP, Donazar JA. Vultures and livestock: the where, when, and why of visits to farms. *Animals* 2020;10(11):2127.
- [9] Margalida A, Campión D, Donazar JA. Scavenger turned predator: European vultures' altered behaviour. *Nature* 2011;480(7378):457.
- [10] Mateo-Tomás P, Olea PP, López-Bao JV. Europe's uneven laws threaten scavengers. *Science* 2018;360(6389):612–3.

- [11] Berzi D, Cerri J, Musto C, Zanni MA. Use of European funds and ex post evaluation of prevention measures against wolf attacks (*Canis lupus italicus*) in the Emilia-Romagna Region (Italy). *Animals* 2021;11(6):1536.
- [12] Nyhus P, Fischer H, Madden F, Osofsky S. Taking the bite out of wildlife damage: the challenges of wildlife compensation schemes. *Conserv Pract* 2003;4:37–43.
- [13] Dabbs GR, Martin DC. Geographic variation in the taphonomic effect of vulture scavenging: the case for Southern Illinois. *J Forensic Sci* 2012;58:S20–5.
- [14] Keyes CA, Myburgh J, Brits D. Animal scavenging on pig cadavers in the Lowveld of South Africa. *Forensic Sci Int* 2021;327:110969.
- [15] Schultz JJ, Mitchell AR. Avian scavenging of small-sized pig carcasses in Central Florida: utilizing GIS to analyze site variables affecting skeletal dispersal. *J Forensic Sci* 2017;63(4):1021–32.
- [16] Spradley MK, Hamilton MM, Giordano A. Spatial patterning of vulture scavenged human remains. *Forensic Sci Int* 2012;219(1–3):57–63.
- [17] Guangdi SI, Dong Y, Wang L, Zhang ZH. Shape similarities and differences in the skulls of scavenging raptors. *Zool Sci* 2015;32(2):171–7.
- [18] Griffin D. Field necropsy of cattle and diagnostic sample submission. *Vet Clin North Am Food Anim Pract* 2012;28(3):391–405.
- [19] Marchitelli B. The pathophysiology of dying. *Vet Clin North Am Small Anim Pract* 2020;50(3):513–24.
- [20] Parai JL, Milroy CM. The utility and scope of forensic histopathology. *Acad Forensic Pathol* 2018;8(3):426–51.
- [21] Caniglia R, Fabbri E, Mastrogioseppe L, Randi E. Who is who? Identification of livestock predators using forensic genetic approaches. *Forensic Sci Int Genet* 2013;7(3):397–404.
- [22] Caniglia R, Galaverni M, Delogu M, Fabbri E, Musto C, Randi E. Big bad wolf or man's best friend? Unmasking a false wolf aggression on humans. *Forensic Sci Int Genet* 2016;24:e4–6.
- [23] Duriez O, Descaves S, Gallais R, Neouze R, Fluhr J, Decante F. Vultures attacking livestock: a problem of vulture behavioural change or farmers' perception? *Bird Conserv Int* 2019;29(3):437–53.
- [24] Margalida A, Campión D, Donazar JA. Vultures vs livestock: conservation relationships in an emerging conflict between humans and wildlife. *Oryx* 2014;48:172–6.
- [25] Hagopian RV. Black vultures and live prey. *Auk* 1947;64:132.
- [26] Lovell HB. Black vultures kill young pigs in Kentucky. *Auk* 1947;64:131–2.
- [27] Sprunt Jr A. Predation of living prey by the black vulture. *Auk* 1946;63:260–1.
- [28] De Toledo LM, Da Costa MP, Schimidek A, Jung JE, Cirylo JNSG, Cromberg VU. The presence of black vultures at the calving sites and its effects on cows' and calves' behaviour immediately following parturition. *Animal* 2013;7(3):469–75.
- [29] Umberger SH, Geyer LL, Parkhurst JA. Addressing the consequences of predator damage to livestock and poultry. Virginia Cooperative Extension; 2009. Retrieved from: <https://vtechworks.lib.vt.edu/bitstream/handle/10919/50717/410-030.pdf?sequence=1&isAllowed=y>.
- [30] Avery ML, Cummings JL. Livestock depredations by black vultures and golden eagles. *Sheep Goat Res J* 2004;19:58–63.
- [31] Ballejo F, Plaza PI, Lambertucci SA. The conflict between scavenging birds and farmers: field observations do not support people's perceptions. *Biol Conserv* 2020;248:108627.
- [32] Murn C. Observations of predatory behavior by white-headed vultures. *J Raptor Res* 2014;48:297–9.
- [33] Reeves NM. Taphonomic effects of vulture scavenging. *J Forensic Sci* 2009;54(3):523–8.
- [34] Politis A, Vasileiou NGC, Cripps PJ, Liagka DV, Boufis PT, Valasi I, et al. Mortality of dairy sheep during the peri-parturition period: results of a field investigation in Greece. *Animals* 2021;11(8):2172.
- [35] Dagalp SB, Babaoğlu AS, Dogan F, Farzani TA, Alkan F. An assessment of bovine herpes virus 4 as a causative agent in abortions and neonatal death. *Onderstepoort J Vet Res* 2020;87(1):e1–5.
- [36] Henker LC, Lorenzett MP, Fagundes-Moreira R, Dalto AGC, Sonne L, Driemeier D, et al. Bovine abortion, stillbirth and neonatal death associated with *Babesia bovis* and *Anaplasma* sp. infections in southern Brazil. *Ticks Tick Borne Dis* 2020;11(4):101443.
- [37] Jawor P, Król D, Mee JF, Sołtysiak Z, Dzimira S, Larska M, et al. Infection exposure, detection and causes of death in perinatal mortalities in Polish dairy herds. *Theriogenology* 2017;103:130–6.
- [38] Mee JF, Jawor P, Stefaniak T. Role of infection and immunity in bovine perinatal mortality: part 1. Causes and current diagnostic approaches. *Animals* 2021;11(4):1033.
- [39] Mickelsen WD, Evermann JF. In utero infections responsible for abortion, stillbirth, and birth of weak calves in beef cows. *Vet Clin North Am Food Anim Pract* 1994;10(1):1–14.
- [40] Mock T, Mee JF, Dettwiler M, Rodriguez-Campos S, Hüslér J, Michel B, et al. Evaluation of an investigative model in dairy herds with high calf perinatal mortality rates in Switzerland. *Theriogenology* 2020;148:48–59.
- [41] Bellows RA, Patterson DG, Burfening PJ, Phelps DA. Occurrence of neonatal and postnatal mortality in range beef cattle. II. Factors contributing to calf death. *Theriogenology* 1987;28(5):573–86.
- [42] Cuttance E, Laven R. Perinatal mortality risk factors in dairy calves. *Vet J* 2019;253:105394.
- [43] Townsend HGG. Environmental factors and calving management practices that affect neonatal mortality in the beef calf. *Vet Clin North Am Food Anim Pract* 1994;10(1):119–26.

- [44] Zaborski D, Grzesiak W, Szatkowska I, Dybus A, Muszynska M, Jedrzejczak M. Factors affecting dystocia in cattle. *Repr Dom Anim* 2009;44(3):540–51.
- [45] Azzam SM, Kinder JE, Nielsen MK, Werth LA, Gregory KE, Cundiff LV, et al. Environmental effects on neonatal mortality of beef calves. *J Anim Sci* 1993;71(2):282–90.
- [46] Blanchard PC. Diagnostics of dairy and beef cattle diarrhea. *Vet Clin North Am Food Anim Pract* 2012;28(3):443–64.
- [47] Caffarena RD, Casaux ML, Schild C, Fraga M, Castells M, Colina R, et al. Causes of neonatal calf diarrhea and mortality in pasture-based dairy herds in Uruguay: a farm-matched case-control study. *Braz J Microbiol* 2021;52(2): 977–88.
- [48] Rousseaux CG. Congenital defects as a cause of perinatal mortality of beef calves. *Vet Clin North Am Food Anim Pract* 1994;10(1):35–51.
- [49] Smith D. Field disease diagnostic investigation of neonatal calf diarrhea. *Vet Clin North Am Food Anim Pract* 2012;28(3):465–81.
- [50] Funnell BJ, Hilton WM. Management and prevention of dystocia. *Vet Clin North Am Food Anim Pract* 2016;32(2):511–22.
- [51] Mee JF, Sanchez-Miguel C, Doherty M. Influence of modifiable risk factors on the incidence of stillbirth/perinatal mortality in dairy cattle. *Vet J* 2014;199(1):19–23.
- [52] Cabrera VE, Fricke PM. Economics of twin pregnancies in dairy cattle. *Animals* 2021;11(2):552. <https://doi.org/10.3390/ani11020552>.