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INVESTIGATION ON SHEEP FARM CHARACTERISTICS, WOLF PREDATION AND ANIMAL WELFARE IN THE GROSSETO PROVINCE (ITALY)

Abstract - C. RUSSO, F. CECCHI, P.A. ACCORSI, N. SCAMPUDDU, M.N. BENVENUTI, L. GIULIOTTI, *Investigation on sheep farm characteristics, wolf predation and animal welfare in the Grosseto province (Italy).*

In Italy, and particularly in Tuscany, the presence of the wolf (*Canis lupus* L.) is steadily increasing causing problems connected with the coexistence with animal husbandry.

In the Grosseto province, the complaints and protests of farmers are particularly strong so, it seemed interesting to evaluate the occurrence of predation events, the welfare level of the sheep farms and their relation with the chronic stress.

The safeguard of animal welfare represent an important topic also in relation with the awareness of the consumers towards the ethic of animal production system.

Sixteen farms in the Grosseto province were investigated. Animal welfare was estimated by the TOS-OVI method; it takes into account five Areas of interest (A: management; B: structures and housing conditions; C: environmental control; D: feeding and watering; E: health and behaviour) giving a final welfare score and judgment.

Farms characteristics for the applying of the TOS-OVI method were gathered. Descriptive analysis of the farms characteristics was performed; one way ANOVA test was used to analyse the variability of cortisol related to the level of welfare and to the animals that experienced (Preyed) or not predation events (Not preyed).

The checked farms were family business of medium-large size and reared Appenninica, Sarda, and Massese breeds. Seven farmers declared at least one predation event in the last six months, with four killed animals on average. Farmers complained not only dead animals but also abortion and decrease in milk production. Sixty-seven percent of the attacks occurred in the morning or in the early afternoon when the flock was free grazing on the pasture.

Results on animal welfare showed an acceptable level in each farm, with optimal results mostly related to health, hygiene and behavioural aspects (Area E), while the Area A, related to management features showed the more critical deficiencies. Hair cortisol was low in all the animals, and it resulted significantly lower in the farms with the highest level of welfare. Differences on hair cortisol between animals that experienced or not predation events displayed significant lower level in preyed ones ($p = 0.0014$). Anyway, there are only few researches on the topic, so it would be interesting to extend the study area for the purpose of deepening the knowledge on this topic.

Key words - sheep, wolf, animal welfare, hair cortisol, Grosseto province, Italy

Riassunto - C. RUSSO, F. CECCHI, P.A. ACCORSI, N. SCAMPUDDU, M.N. BENVENUTI, L. GIULIOTTI, *Indagine preliminare sulle caratteristiche aziendali, la predazione da lupo e il benessere animale in allevamenti ovis della provincia di Grosseto (Italia).*

La presenza del lupo è sempre più diffusa in Italia ed in Toscana in particolare, causando pertanto problemi alle aziende ovi-caprine di tipo estensivo presenti sul territorio, con perdite economiche notevoli per gli allevatori che talvolta, proprio per questo motivo, cessano la loro attività. Nel presente studio l'attenzione è stata rivolta alla provincia di Grosseto dove le proteste degli allevatori sono particolarmente forti. La ricerca ha valutato in sedici allevamenti l'entità della predazione da lupo, il livello di benessere degli ovis attraverso un metodo ad indice (TOS-OVI) e il livello di cortisolo nel pelo come indicatore di stress cronico. Il metodo di valutazione del benessere animale prende in considerazione cinque Aree (A: management; B: strutture e condizioni di allevamento; C: controllo ambientale; D: alimentazione e acqua di bevanda; E: salute e comportamento) fornendo un punteggio finale e un giudizio di merito per ogni Area e per l'azienda nel suo complesso.

È stata effettuata un'analisi descrittiva delle caratteristiche aziendali e del livello di benessere nelle aziende e nelle singole Aree; è stata utilizzata l'analisi della varianza per valutare le differenze del livello di cortisolo sia in relazione alla Classe di benessere ottenuta dalle aziende, sia tra gli animali che hanno subito o meno l'esperienza della predazione.

Le aziende erano a conduzione familiare e di dimensioni medio-grandi; le razze allevate erano Appenninica, Sarda e Massese. Sette allevatori hanno dichiarato almeno un evento predatorio e una media di quattro capi uccisi negli ultimi sei mesi. Gli allevatori oltre a lamentare la morte degli animali segnalano anche aborti e diminuzione di latte come conseguenza dell'evento predatorio. Il 67% degli attacchi è avvenuto durante la mattina o il primo pomeriggio, quando gli animali sono al pascolo. I risultati relativi alla valutazione del benessere animale hanno mostrato livelli accettabili in tutte le aziende con punteggi ottimali soprattutto per gli aspetti legati alla salute, all'igiene e al comportamento animale (Area E), mentre quelli più critici sono emersi nella Area A che si riferisce agli aspetti manageriali.

I valori di cortisolo del pelo sono risultati contenuti in tutti gli animali e significativamente più bassi nelle aziende con livello di benessere più elevato. Il valore di cortisolo in animali che hanno avuto esperienza di predazione è risultato significativamente più basso di quelli non predati ($p = 0.0014$).

Questa è una ricerca preliminare per cui sarebbe interessante ampliare lo studio per approfondire le conoscenze vista la scarsità di ricerche su questi aspetti.

Parole chiave - ovis, lupo, benessere animale, cortisolo del pelo, provincia di Grosseto

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INTRODUCTION

In Tuscany sheep breeding is mainly based on semi-extensive system that imply living outdoor most of the time; this fact, along with the rusticity and frugality of these animals, is perceived as guarantee of the well-being of animals (Goddard *et al.*, 2006). However, this perception does not consider that these extensive farming systems are not completely able to prevent the various aversive occurrences that can repeatedly expose the animals to stress (Destrez *et al.*, 2013). In fact, animal welfare is a multidimensional concept that comprises not only physical but also psychological aspects (De Vries, 2015).

The losses of livestock derived to depredation are of primary concern, but at the same time, stress induced in livestock exposed to depredation threat is likewise alarming. Emotional experiences, such as wolf attack, can threaten animal welfare and productivity with deleterious consequences on farm profitability (Webber *et al.*, 2015). Otherwise, it is also known that, from an evolutionary perspective, defensive reactions promote fitness in wild animals and let to avoid sources of danger such as predators (Forkman *et al.*, 2007).

By the end of the nineteenth century, because of persecution and changes to its habitat, the grey wolf (*Canis lupus* L.) became extinct in many European countries (Ciucci *et al.*, 2005; Espuno *et al.*, 2004; Iliopoulos *et al.*, 2009; Lanszki *et al.*, 2012). Nowadays, the wolf population in Italy and in many other country of Europe is in an expansion phase leading to the re-colonization of large areas in the Apennines and Alps. This trend is reinforced tanks to the strong aptitude of wolf for recolonization once persecution was stopped (Chapron *et al.*, 2003). Generally, wolves inhabit regions away from human activities including husbandry (Boitani, 2000), but even due to the raise in wolf numerousness, increases the conflict with human particularly in consequence of livestock predation (Chapron *et al.*, 2003; Gula, 2008; Iliopoulos *et al.*, 2009; Pimenta *et al.*, 2017). Based on several studies carried out in different areas of Italy, sheep is the species most preyed upon the wolf, followed by cattle and goats (Gazzola *et al.*, 2008). Cattle is the specie most preyed in Portugal and in Spain, while goat is the most preyed in Greece; in Poland and Hungary the main preys of wolves were red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), and wild boar (*Sus scrofa*) (Pimenta *et al.*, 2017).

The analysis of conflict between farmers and predators, the importance of identifying livestock husbandry systems in each country and technical solution for mitigating this problem is essential even for wildlife conservation (Arranz Sanz, 2005).

In particular, in Italy, after the dramatic reduction of wolf population size and range during the 1950s and the 1960s (Ciucci & Boitani, 1998), the presence noticeably increased (particularly in Tuscany) thanks to the species protection by Italian and International laws.

Therefore, wolf predations represent a focal problem for sheep farms based on free grazing rearing, causing significant economic losses to farmers who, sometimes, decide to close their activity (Dondina *et al.*, 2015). These problems are profoundly felt in Europe and particularly in the South, where livestock depredation is crucial for human activities preservation (Iliopoulos, 2009).

In the past, investigations on wolf predation have been carried out in some provinces of Tuscany (Pisa, Lucca, and Arezzo) (Gazzola *et al.*, 2008; Mattiello *et al.*, 2010; Mattiello *et al.*, 2012; Novak *et al.*, 2011; Russo *et al.*, 2013; Russo *et al.*, 2014); in this study, the attention was turned on the Grosseto province where the complaints and protests of farmers are particularly strong.

In this area sheep rearing is mainly based on semi-extensive system and this is perceived as guarantee of the well-being of animals (Gastaldo *et al.*, 2015), on the basis of the freedom to express the behavioural patterns, without taking into account other aspects such as the emotional and physical consequences of wolf predation.

Several are the systems for evaluating animal welfare: some of these are founded on behavioural, health, and physiological measures taken directly on the animal (animal based systems), others are founded on parameters related to farm structure and management characteristics (resource based systems) (Main *et al.*, 2003). The “on farm” welfare assessments are mainly based on the latter because they mostly take into account objective, repeatable and easily measurable parameters. A group of researchers belonging to the Department of Veterinary Science of Pisa developed a resource based method named TOS-OVI (Gastaldo *et al.*, 2015) used to evaluate the welfare level in Tuscan sheep farms.

Cortisol is considered as a biomarker of stress conditions and its measurement in hair matrix allows detecting factors leaded by long-term variations ascribable to chronic unsafe living conditions. The measurement of glucocorticoids can reveal how animals perceive and adapt themselves to their environment.

In the present study, the farms characteristics, the occurrence of the wolf predatory events, and the animal welfare level in farms located in the Grosseto province were described. The aim of the study was to analyse the influence of the experience of predation and the “on farm” welfare level of the sheep farms on the chronic stress useful to arrange suitable strategies of prevention.

MATERIALS AND METHODS

The on-farm surveys were carried out in sixteen farms in the Grosseto province, located in Tuscany. This Province is a coastal area of the southern end of Tuscany (42°46'20"N 11°06'32"E) that comprises 4.504 km². This province extends from the Tyrrhenian coast in the west to the slopes of the volcanic Amiata massif in the east, encompassing a great variety of habitats and a wide altitudinal range such as the slopes of the Colline Metallifere and the marshland of Maremma (Ciucci & Boitani, 1998) (Fig. 1).

We focused our study only on farms situated in hilly areas, where the presence of wolf has been documented (Mattiello *et al.*, 2010). Hilly areas are in large part suitable for grazing and for this reason, a great number of sheep farms is present.

Farms with a flock size smaller than 100 animals were excluded from the sample because they are family-run businesses with very simple farming systems. The breeders were previously contacted to request availability to undergo the interview and then an on-farm survey was performed by a trained researcher. Information about farming structure and wolf predation were collected by a check list previously used in similar studies on this topic in order to assess the relevance of the predation, the temporal distribution of predation events and the prevention methods (Mattiello *et al.*, 2010; Mattiello *et al.*, 2012; Russo *et al.*, 2013; Russo *et al.*, 2014).

Animal welfare level was performed by TOS-OVI index method; it consists of three tools: a check list, a technical file and an Excel spreadsheet. The check list, based on restricted, objective and easily measurable parameters, defines farm's characteristics (Gastaldo *et al.*, 2015). In accordance with European regulation (EC, 2006), the check list was divided in five Areas: A. Farm management and staff; B. Farm structures and housing conditions; C. Environmental control; D. Feeding and watering; E. Health management and behavior (Appendix 1). The check list was supported by a technical file that describes all the examined parameters and assigns them a score (positive or negative) in relation to its repercussion to animal welfare. This system assigned a higher score to the aspects of greater relevance and of more objective evaluation and a lower score to the parameters influenced by the time of survey and more subjective. The Excel spreadsheet automatically assigned both a partial score to each Area and a total farm score. Depending on the score, Areas, as well as the whole farm, were evaluated according to five classes of increasing level of welfare corresponding to a judgment: 1 = scarce; 2 = sufficient; 3 = moderate; 4 = good; 5 = excellent.

For cortisol analysis, hair samples were carefully cut from the tail switch using clippers and were frozen to -20°C to prevent lice, which are often found in this

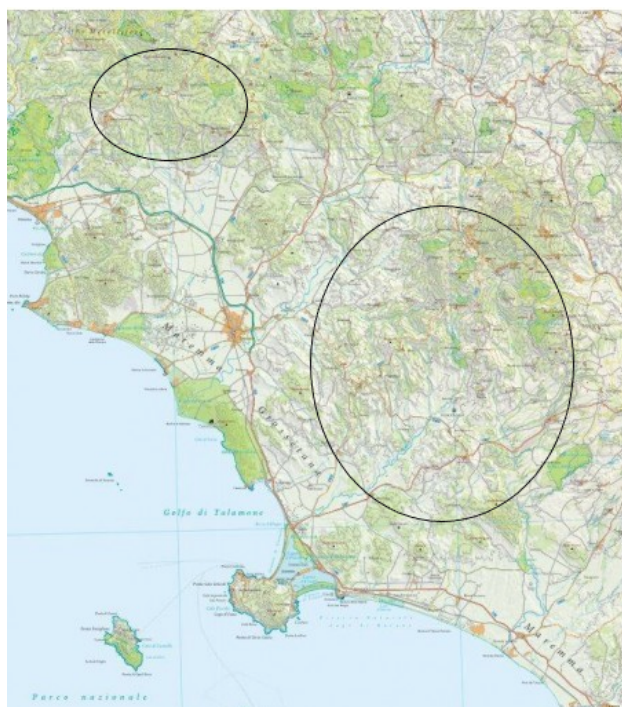


Figure 1 - Location of the inspected districts.

body area. The analyses were carried out following Accorsi *et al.* (2008) method.

Descriptive analysis of the farms characteristics was performed; one way ANOVA test was used to analyse the variability of cortisol related to the level of welfare and T test was applied to point out the differences among data. ANOVA test was also used to verify statistical differences in cortisol level in animals that experienced (Preyed) or not predation events (Not preyed): the preyed animals are not subjected to any kind of injury but they are frightened and stressed for the attack by wolves (Bruns *et al.*, 2020).

Data were processed by the JMP software (JMP., 2007). Differences were considered significant for p-values < 0.05.

All the procedures were carried out in compliance with the current legislation on protection of animals used for scientific purposes (Directive 2010/63/EU).

RESULTS AND DISCUSSION

The checked farms were of medium/large size with a mean extension of 65 ± 73 ha. The number of heads per farm varies from 103 to 2575 with a mean value of 427 (S.E. = 149.43). Forty-four percent of the farms reared Appenninica breed, the others reared Sarda (37%) and Massese breed (19%). Six farms reared only sheep, the others also goats, cows and horses.

“Appennica” sheep originated from the Appennines. It is of medium-large size, polled in each sex with white fleece and slightly curved face profile. Its aptitude is mainly addressed to meat production, with heavy lambs obtained in short time.

“Sarda” sheep originated from Sardinia but nowadays it is widespread throughout central and southern Italy as well as in other Mediterranean countries, thanks to its adaptability. It is a medium-size breed with white fleece. Rams are occasionally horned and ewes are polled. Sarda sheep is the major Italian breed addressed to milk production that is mainly transformed in typical cheeses.

“Massese” sheep originated from the province of Massa-Carrara and is reared especially in Tuscany but also in Liguria, Umbria, and Lazio. It is of medium-large size with dark fleece and spiral horns. It is characterised by a light but sturdy skeleton, big abdomen, and large udder. Its production is mainly milk but it is appreciated also for the lamb meat.

All farms were family-run businesses, with a number of employees ranging from one to five. In accordance with the law, the animals were checked daily; 43% of the farms have employees that attended refresher courses on farming related subject.

Fifty-two percent of farms were equipped with automatic systems mostly represented by milking machines. Data evidenced that all Sarda breeders used automated milking systems and their staff showed a higher willingness towards enhancing farm practices. However, breeders not specializing in dairy sheep did not use that type of milking equipment.

Sheep grazed on turned pasture in most cases and farmers provided feed integrations to the animals especially during the harsh and dry seasons; water supply on pasture was provided in 64% of the farms.

All breeders were aware of the presence of wolf, that represents a problem for animals living outdoor for most of the time. The assessment of the wolf presence is mainly based on direct sighting or on presence signs: howlings, faeces and tracks. Most farmers (49.5%) considered that the main defence measure should be the elimination of wolves; 44% would like to revise the legislation, while the remaining (6.5%) furnished both answers, moreover requesting to relocate wolves to other areas, in light of the incompatibility of wolf with human activity.

Only 25% of the farms were provided with anti-wolf fences of an appropriate height (180-220 cm) and made of electro-welded mesh (Berzi, 2014). On the other hand, all farms had closed shelters that sheep use for resting during the night, during the day when the weather is bad (69%) and for milking procedures (56%).

Nine farms had livestock guardian dogs in a sufficient number (1:130): in fact the advisable number of

dogs required for the flock varies along with several factors such as the size of pasture, the number of animals, the typology of the environment, the number and species of predators but, in general, the recommendation is one dog per 100-150 ewes (Mattiello *et al.*, 2012).

The guardian dogs belonged to the typical breeds suitable for this purpose: Maremmano, Caucasian and Pyrenees shepherds. No farmer had taken out an insurance policy against predation damages that mostly consist in the death of ewes or lambs; unfortunately, in addition to the murdered animals, breeders complained about abortion and decrease in milk production due to fear and stress (Laporte *et al.*, 2010). In seven farms (44%), farmers reported one predation event in the last six months, with 4.28 ± 4.68 killed animals on average and a range from 1 to 14. As already observed in other provinces, 67% of the attacks occurred in the morning or in the early afternoon when the flock is grazing free on the pasture; during the night, the animals are closed in shelters and the wolf has consequently modified its predatory behaviour (Russo *et al.*, 2018). In light of the present survey and of those carried out in previous years (Mattiello *et al.*, 2010; Mattiello *et al.*, 2012; Russo *et al.*, 2014) it is possible to state that it would be advisable to adopt more preventive measures to assure the coexistence between human activity and ecosystem safeguard.

Regarding farms characteristics, information gathered during the survey are reported in Tab. 1.

Data regarding the distribution of farms in the five classes of animal welfare showed that the 55% of these reaches a moderate level of welfare (Class 3) in accordance with previous study carried out in Tuscany (Gastaldo *et al.*, 2015). None of the farms was in Class 1 and in Class 4 and only 18% of farms reached the highest level of welfare, demonstrating the need to improve the care towards the animals; the remaining part was classified as sufficient (Class 2).

The total score for the whole farm corresponded to Class 3 (“moderate”). In particular, Area A, concerning management practices, presented more deficiency (Class 1) due to the poor level of mechanization, to the insufficient attendance to refresher courses and to the lack of effective prevention methods of attacks by predators, as previously described. Area E (health, hygiene and behavioural aspects) recorded the highest score thanks to the presence of a health plan in almost all farms. All the scores for each area were presented in Fig. 2.

Hair cortisol level in relation with the TOS-OVI score revealed significant differences ($df = 2$; $F = 3.326$; $p < 0.041$) (Tab. 2); this fact was more evident in the group with Class 5 where the effective management strategies can contribute to maintain lower level of stress of the animals.

Table 1. Parameters detected in the 16 surveyed farms.

| Parameters | |
|---|-------------|
| Presence of automatic systems (%) | 52 |
| Farmer's training (%) | 43 |
| Practice of tail docking (%) | 45 |
| Practice of hear docking (%) | 27 |
| Shearing/year (%) | 100 |
| Sudden weaning (%) | 45 |
| Progressive weaning (%) | 55 |
| Weaning after five weeks of age (%) | 100 |
| Stocking rate on pasture (UBA/ha) (mean ± S.D.) | 1.04 ± 0.51 |
| Indoor space allowance (m ²) (mean ± S.D.) | 519 ± 999 |
| Presence of structures for isolation (%) | 82 |
| Presence of structures for lambing (%) | 64 |
| Presence of roof insulation (%) | 27 |
| Presence of shelter on pasture (%) | 100 |
| Presence of expert in animal nutrition (%) | 54 |
| Practice of feed analysis (%) | 54 |
| Rotational grazing system (%) | 82 |
| Grain integration (%) | 18 |
| Concentrate supplementation (%) | 64 |
| Potable water supply (%) | 91 |
| Water availability on pasture (%) | 64 |
| Additional drinker in summer (%) | 45 |
| Presence of straw bedding (%) | 100 |
| Frequency of bedding removal/year | 2.18 ± 0.7 |
| Frequency of folder disinfections/year (mean ± S.D.) | 2.6 ± 0.8 |
| Presence of an health plan (%) | 64 |
| Parasites monitoring, treatments only in effective case (%) | 27 |
| Presence of flies and rats control plan (%) | 100 |
| Quarantine (%) | 45 |

Table 2 - Hair cortisol level related to the TOS-OVI class of welfare.

| Class of welfare | Cortisol (pg/mg) | | |
|------------------|------------------|-------|-------|
| | n | Mean | S.E. |
| 1 | 0 | - | - |
| 2 | 21 | 0.12a | 0.006 |
| 3 | 42 | 0.16a | 0.018 |
| 4 | 0 | - | - |
| 5 | 17 | 0.07b | 0.029 |

Different letters show significant differences.

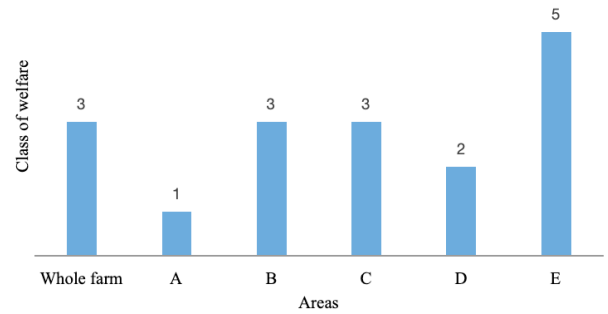


Figure 2 - Classes of welfare (TOS-OVI score) for the farm and for each Area. A. Farm management and staff; B. Farm structures and housing conditions; C. Environmental control; D. Feeding and watering; E. Health management and behaviour. Class of welfare: 1 = scarce; 2 = sufficient; 3 = moderate; 4 = good; 5 = excellent.

Hair cortisol was low in all the animals (Ghassemi Nejad *et al.*, 2014), and it resulted significantly lower in the farms with the highest level of welfare. Differences in hair cortisol between animals that experienced or not predation displayed a controversial result (Tab. 3): in fact, the preyed ones exhibited significantly lower ($p = 0.0014$) hair cortisol level, indicating the lack of long term stress conditions. Anyway, it is ascertained that fear of potential predators led to antipredator strategies, which are based mainly on associations between some aspects in the animal's environment and adaptive strategies (Boissy, 1998).

Table 3 - Hair cortisol level related to predation events.

| Animal | Cortisol (pg/mg) | | |
|------------|------------------|------|-------|
| | n | Mean | S.E. |
| Not preyed | 42 | 0.17 | 0.018 |
| Preyed | 38 | 0.09 | 0.019 |

However, the cortisol levels did not reach worrying values in each group. We hypothesize, as reported by Forkman *et al.* (2007), that the reaction to fear is connected not only with the appearance of the event but also with other characteristics, as novelty, intensity, duration, suddenness or proximity that were not possible to ascertain in our study and that could have affected the results.

In any case, a better management of the livestock should conciliate the wolf conservation and the urgency of maintaining the human activities (Dondina, 2015; Bruns *et al.*, 2020).

CONCLUSION

The present study confirms that wolf is widely spread in the Grosseto province and the existence of a high conflict between the presence of the wolf and husbandry: so, an effective management of the wolf should conciliate the species conservation and the human activities. As regards animal welfare, in general, the results are acceptable for the whole farm, with optimal results for the Area E (health, hygiene and behavioural aspects). Hair cortisol showed controversial results in preyed and not preyed animals; however, cortisol did not reach high levels in all animals.

This is one of the first research on the topic, so it would be interesting to gain further insight with additional investigations.

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APPENDIX 1

Scored parameters included in each Area of the checklist.

| Area | Parameters |
|---|---|
| A. Farm management and staff | Farmer's training Frequency of animal inspection Annual control of each automatic equipment Acquaintance of milking machine parameters Frequency of inspection of milking machine Tail docking Age of tail docking and length of the stamp Hear docking Dehorning Frequency of shearing Frequency of feet control Weaning Age of weaning Precautions from predation Implemented precautions Efficacy of implemented precautions Presence of fences and their characteristics Presence of shelters and their characteristics Detection of wolf signs of presence Presence and characteristics of guardian dogs Frequency of predation Number of preyed animals Time of predation |
| B. Farm structures and housing conditions | Stocking rate on pasture Indoor space allowance Presence of hazards Condition of feeders and equipment Structures for isolation Structures for lambing |
| C. Environmental control | Lighting and ventilation Artificial lighting Roof insulation Cooling techniques Odours into the sheepfold Shelter on pasture |
| D. Feeding and watering | Presence of expert in animal nutrition Frequency of feed analysis Pasture management Feed integration Cleanliness of equipment Water supply: type of water, frequency of analysis for non-potable water, presence of water purification system, presence of water storage. Number and type of drinker Water availability on pasture Cleanliness of drinkers Additional drinker in summer |
| E. Health management and behaviour | Frequency of bedding removal Frequency of folder disinfections Presence of an health plan Frequency of monitoring of hygienic parameters of milk Problem solving in case of somatic cells count over the threshold Frequency of overcoming somatic cells count threshold Parasites monitoring, treatments only in effective case Presence of flies and rats control plan Quarantine Appearance, cleanliness and behaviour of animals |

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