Elsevier Editorial System(tm) for Small Ruminant Research Manuscript Draft

Manuscript Number: Rumin-D-11-3643R2

Title: Sheep predation: Characteristics and risk factors

Article Type: Research Paper

Keywords: Predation; Canis lupus; Large carnivores; Sheep; Wildlife conflict

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Manuscript Region of Origin: ITALY

Abstract: Predation has always been an important problem in extensive sheep farms, causing serious economic losses to the farmers. Official predation reports have recently been decreasing in the District of Pisa, in spite of the presence of two wolf packs in the area. The aim of the present research was to obtain reliable information on the characteristics of predation and to estimate the effectiveness of existing prevention methods in sheep farms of the southern District of Pisa, in order to set up predictive models for an improved and more focused prevention plan and support interventions by public authorities. On-farm surveys were carried out in 73 semi-extensive sheep farms. Predation events were reported by 75.3% of the farmers. Wolves seemed to be responsible for most of those events, although their actual role could be confirmed only in 34% of cases. Most of the events occurred in spring and 85.1% of them were concentrated during night time. The average number of sheep killed during each attack was 7.05. In 22.3% of cases, the number of sheep killed was \geq 10. Proximity to protected areas and the presence of thick vegetation cover significantly affected the probability of a farm being subjected to chronic predation. Farm size was significantly higher in those cases. No clear indication about the effectiveness of prevention methods could be obtained from our survey. The results of this investigation highlighted the impact of predation in the Southern District of Pisa and emphasized the need for finding technical and political solutions to this problem. Attention should be focused on large farms, with thick vegetation cover and located close to protected areas. Further investigations should be carried out in order to test the effectiveness of suitable prevention methods in these farms.

The title and the running title have been changed, following the reviewer's suggestion.

The manuscript (text, figures and tables) has been reviewed again from a native English speaker. We hope that there are no more errors in this new version. All the changes are highlighted in red.

Thank you for the quick revision and the useful comments.

Best regards

Silvana Mattiello & co-authors

1	Running title: Sheep predation in Italy
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4	Original Research Paper
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7	Sheep predation in the Southern District of Pisa (Italy):
8	Characteristics and risk factors
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1 Abstract

2 Predation has always been an important problem in extensive sheep farms, causing serious 3 economic losses to the farmers. Official predation reports have recently been decreasing in the 4 District of Pisa, in spite of the presence of two wolf packs in the area. The aim of the present 5 research was to obtain reliable information on the characteristics of predation and to estimate the 6 effectiveness of existing prevention methods in sheep farms of the southern District of Pisa, in order 7 to set up predictive models for an improved and more focused prevention plan and support 8 interventions by public authorities. On-farm surveys were carried out in 73 semi-extensive sheep 9 farms. Predation events were reported by 75.3% of the farmers. Wolves seemed to be responsible 10 for most of those events, although their actual role could be confirmed only in 34% of cases. Most 11 of the events occurred in spring and 85.1% of them were concentrated during night time. The 12 average number of sheep killed during each attack was 7.05. In 22.3% of cases, the number of 13 sheep killed was ≥ 10 . Proximity to protected areas and the presence of thick vegetation cover 14 significantly affected the probability of a farm being subjected to chronic predation. Farm size was 15 significantly higher in those cases. No clear indication about the effectiveness of prevention 16 methods could be obtained from our survey. The results of this investigation highlighted the impact 17 of predation in the Southern District of Pisa and emphasized the need for finding technical and 18 political solutions to this problem. Attention should be focused on large farms, with thick 19 vegetation cover and located close to protected areas. Further investigations should be carried out in 20 order to test the effectiveness of suitable prevention methods in these farms.

21

22 Key words: Predation, *Canis lupus*, Large carnivores, Sheep, Wildlife conflict.

23

1 Introduction

2 Predation has always been an important problem in extensive sheep farms, causing serious 3 economic losses to the farmers (Muhli and Musiani, 2009). In recent years, this problem has been 4 spreading along the Italian Apennines, and is becoming an important issue also in the District of 5 Pisa (AA.VV., 2007). Currently, in this District, predation represents the main cause of mortality in 6 48% of the sheep farms and the presence of wolves in the area is perceived as a problem by 86.3% 7 of the farmers. Predation events occur regularly in many farms, although they are seldom officially 8 reported to the Provincial Authority, as compensations are not considered adequate and farmers are 9 obliged to sustain additional costs for carcass destruction in order to comply with the Regulation EC 10 1774/2002 (the "Animal By-products Regulation") (Mattiello et al., 2010). The number of 11 complaints for predation to the Pisa Provincial Authority has been decreasing since Regulation EC 12 1774/2002 was enacted, passing from a peak of nearly 30 predation events in 1999 to only 2 events 13 reported (and verified by State veterinarians) in 2004 (AA.VV., 2007). A similar trend has been 14 observed in the District of Florence, with a further decrease after 2005, following the adoption of 15 the Regional Law n. 26/2005, which eliminates the possibility of direct reimbursements to the 16 farmers and sets up the obligation of insurance for access to reimbursements (Berzi et al., 2008). 17 The additional costs charged to farmers (for carcass destruction and insurance) are probably 18 responsible for the reduction of official complaints of predation events. The lack of official 19 information on these events represents a major obstacle to complete knowledge of the phenomenon 20 and therefore to its effective control. The analysis of conflict between farmers and presence of 21 predators, and as well as the research of technical and political solutions for mitigating this conflict, 22 are essential not only for farmer protection, but also for wildlife conservation (Ciucci et al., 2005; 23 Espuno et al., 2004; Arranz Sanz, 2005). The conflict with animal husbandry is considered a major 24 threat to the conservation of some important predator species and it is one of the main factors which 25 has lead wolves close to extinction (Ciucci et al., 2005).

1 The present investigation was aimed at obtaining reliable information on the characteristics of 2 predation in sheep farms of the southern District of Pisa, in order to set up of predictive models for 3 an improved and more focused prevention plan (Musiani et al., 2005) and to support interventions 4 by public authorities. To this aim, we investigated the cCharacteristics (in terms of number and 5 frequency of predation events, predator's identity and number of sheep killed during each attack) 6 and the temporal distribution of predation events (frequency of occurrence and year, season and 7 time of occurrence) were investigated in order to describe their dynamics and to understand the 8 importance entity of the problem. Furthermore, we tried to identify the main management (e.g. farm 9 size, sheep/stockman ratio) and environmental risk factors (e.g. farm location and vegetation cover) 10 were described in order to understand which farms could be considered at higher risk of predation. 11 Finally, we collected information about the presence of prevention methods and, when they were 12 applied, was collected we tried to estimate their effectiveness, in order to and identify the most 13 rewarding defensive strategies.

14

15 Material and methods

16 The study was carried out in Val di Cecina-(Fig. 1), Southern District of Pisa (Tuscany, Italy). In 17 this area, the stable presence of two wolf packs in the Berignone-Tatti and Monterufoli-Caselli 18 Natural Reserves was confirmed (AA. VV., 2007).

In April-May 2009, on-farm surveys were carried out in 73 semi-extensive sheep farms recruited on the basis of the information obtained by the Farmers' Associations (A.P.A., C.I.A., Coldiretti, Unione Agricoltori) and Local Public Health Service. This sample included almost all the sheep farms located in of the Southern Province of Pisa. The only farms excluded from the survey were those with a flock size smaller than 10 animals and one farm whose farmer did not accept to be interviewed. Flock size ranged from a minimum of 10 to a maximum of 1300 sheep (mean: 339 sheep). Most of the farms were family managed and the farmers lived on the farm.

26 The respect of anonymity was guaranteed to all the farmers that were included in the survey.

Farm locations were placed in regional technical maps and their minimum distance from the
 Berignone-Tatti and Monterufoli-Caselli Natural Reserves was calculated using the software
 ArcView GIS 3.2[©].

4 During the visits, detailed information was collected in order to answer to specific questions. First, 5 in order to assess the importance of the problem, questions were asked on the number and 6 frequency of predation events, on the number of sheep killed during each attack and on the 7 predator's identity as attributed by the farmer and whether the predator's identity had been 8 confirmed otherwise. We were also interested in uUnderstanding the temporal distribution of 9 predation events (frequency of occurrence and year, season and time of occurrence) as these 10 characteristics can assist in confirming the predator's identity and in recommending appropriate 11 prevention strategies. Furthermore, we posed qQuestions about farm size (number of sheep and surface area), sheep/stockman ratio, frequency of presence of the stockman with the sheep and 12 13 environmental factors (vegetation cover and proximity to protected areas) were asked to identify 14 risk factors and to thereafter to be able to predict which farms could be considered at higher risk for 15 predation. Finally, in the survey we asked questions about the presence, type and use of prevention 16 methods (fences or night shelters, guardian dogs or other methods) were asked and, when they were 17 applied, an estimate of their effectiveness was assessed in order to identify the most rewarding 18 defensive strategies.

On the basis of the information collected, farms were assigned to three levels of predation:
"absent", "sporadic" (one event/year), and "chronic" (two or more events/year).

Non parametric analysis of variance (Kruskal-Wallis test) was used to compare continuous variables (distance from protected areas, number of sheep/farm, farm surface area and sheep/stockman ratio), while Chi square test was used to compare frequency distributions of farms affected by absent, sporadic or chronic predation depending on their proximity to protected areas, on the presence of vegetation cover, on the presence of farmers with the sheep, on the use of fences

or shelters or of other prevention methods. All analyses were performed using SPSS 14.0 for
 Windows (SPSS Inc., 2005, Chicago, Illinois).

3

4 **Results**

5 Frequency and characteristics of predation events

In 55 out of 73 farms (75.3%) the farmers reported at least one predation event. Predation was absent in 18 out of 73 farms (24.7%), sporadic in 28 (38.3%) and chronic in 27 (37%). The oldest event went back to 1985, but most of the events (66.7%) occurred after the year 2000. The information about old events was scarce and incomplete. Therefore, attention was focused on events that occurred from 2005 onward. From 2005 until the date of the present survey, 13 (23.6%) of the 55 farms subjected to predation reported one predation event, 26 (47.3%) reported 2-10 events and the remaining 16 (29.1%) reported more than 10 events.

According to the farmers, out of 113 predation events recorded in detail, 103 (91.2%) were due to wolves, three to dogs, two to wild boars and the remaining five to unidentified predators. However, responsibility of wolves could be confirmed (by direct observation or by veterinary inspection) in only 34% (35/103) of the events attributed to wolves by the farmers.

Most of the events attributed to wolves occurred in spring, with a peak in April (Figure 1), and 85.4% of them (88/103) occurred during night time. The average (\pm s.e.) number of sheep killed during each attack was 7.05 \pm 0.80 (min 0, max 37). In 22.3% of cases (23/103), the number of sheep killed was \geq 10. Ewes (77.1%) and lambs (22.2%) were the most frequent preys, while predation on males was almost absent.

22

23 Risk factors

Proximity (within a 5 Km perimeter) to protected areas significantly increased the probability of a farm being subjected to chronic predation (Table 1; p<0.001). The average distance from protected areas was significantly lower in farms subjected to predation (6,890 \pm 744 *vs* 10,716 \pm 1,072 m, 1 with and without predation, respectively; p<0.01), especially if predation was chronic (4,683 \pm 465

2 vs 9,682 \pm 852 m, with chronic and sporadic/absent predation, respectively; p<0.001).

3 The presence of medium/thick vegetation cover also increased the occurrence of chronic predation
4 (Table 2; p<0.01).

Farm size, in terms of number of sheep and surface area, was higher in farms affected by chronic
predation. These farms were also characterized by a higher ratio between the number of sheep and
the number of stockmen (Table 3).

8

9 *Prevention methods*

10 Neither presence of farmers living on the farm, nor constant presence of a stockman with the sheep, 11 or daily animal control procedures reduced the risk of predation. Predation events were reported 12 even in the presence of the farmer, although, in these cases, the farmers commented that the number 13 of sheep killed was lower than during events that occurred in their absence.

14 The only prevention methods adopted were gas guns, anti-wolf night fences, night shelters and 15 guardian dogs. Gas guns were present in only two farms always in combination with other 16 prevention methods, therefore it was not possible to assess their effectiveness.

Day fences were present in almost all of the farms (71 out of 73), while night fences were present only in six farms. No fences were electrified. Their presence did not help to prevent predation, and their characteristics (in terms of height, mesh shape and size, anchorage and depth into the ground) did not affect the occurrence of predation. However, farmers reported a possible effect of anti-wolf night fences to reduce the intensity of predation during the summer, when sheep are usually left grazing at pasture during the night.

The presence of night shelters helped to partially reduce the risk of chronic predation, but only if this procedure was adopted for all animal categories (not only for pregnant females and for lambs) throughout the whole year (not only during the cold season) (p<0.05; Table 4).

1 Guardian dogs were present in 38 farms (52.1% of the farms). The average number of dogs in these 2 farms was 5 ± 0.5 dogs/farm (range: 1-15). The most common breeds were Maremmano, Great 3 Pyrenees and Caucasian shepherds. They were present mainly in large farms (with an average of 4 more than 500 sheep/farm), and the average number of sheep per guardian dog was 119.5 ± 12.0 5 (min 20, max 325). Dogs had been present for more than ten years in 60.5% (23/38) of the farms, 6 whereas in 20.8% (8/38) of the farms they were introduced after 2005. Therefore, a comparison of 7 the effectiveness of guardian dogs before and after their introduction was not possible. The 8 frequency of chronic predation was higher in farms with guardian dogs than in those without 9 (52.6% vs 20%, respectively; p<0.01). However, 27% of the farmers declared that there was a 10 reduction of predation events following the introduction of guardian dogs.

11

12 **Discussion**

13 The impact of predation on sheep farms in the District of Pisa has become quite important during 14 the last decade. This trend is opposite to the official version reported in the Provincial Hunting 15 Management Plan (AA.VV., 2007). This discrepancy highlights the importance of the present 16 survey in obtaining more reliable data for the problem. The wolf is most likely responsible for most 17 predation events. Although in many cases confirmation of responsibility was not demonstrated and 18 could only be presumed, the temporal and spatial distribution of predation events together with their 19 characteristics seem to support the hypothesis of the role of wolf in these events (Zimmerman et al., 20 2007). This is also supported by the fact that the impact of predation has been increasing in parallel 21 with the expansion of wolves in Central Italy. Their presence in Italy was extremely reduced after 22 the II World War (probably no more than one hundred animals) and conservation of this species 23 was considered at risk (Cagnolaro et al., 1974; Zimen and Boitani, 1975). However, over time, a 24 gradual increase of wolf numbers and distribution was observed, especially in Central Apennines 25 (Boitani and Fabbri, 1983; Pandolfi, 1983; Boitani and Ciucci, 1993). In Tuscany the presence of wolves has been confirmed throughout the entire region by several authors (Berzi and Valdrè, 2002; 26

Capitani et al., 2006). As already mentioned, in the District of Pisa several wolf sightings have been
 recorded and the presence of two packs has been located in the Southern part of the District, along
 with a more recent pack in the Central area (Chianni - Santa Luce Mountains) (AA.VV., 2007).

4 The annual peak of predation was recorded in the spring. For wolves, this represents a crucial 5 period, as it corresponds to the birth season and therefore to an increase in nutritional requirements 6 of the pack (Lopez, 1995). Furthermore, the attacks were more frequent during night hours, in 7 agreement with the typical wolf behaviour (Zimmerman et al., 2007). The high number of sheep 8 killed during each attack confirms the presence of surplus killing, which has frequently been 9 reported in wolves on medium sized, vulnerable and abundant preys, such as farmed sheep, 10 especially during the denning period (Lopez, 1995). The high impact of predation on adult animals 11 has also been reported for wolves by other authors (Meriggi and Lovari, 1996), and it is obviously 12 concentrated on females, that represent most of the animals in the farm.

The location of predation events also suggests that wolves play an important role in these events. Most of the farms subjected to chronic predation lie in an area within 5 Km from the two Natural Reserves of Berignone-Tatti and Monterufoli-Caselli, where the presence of two wolf packs has been identified (AA.VV., 2007). Proximity to these areas can therefore be considered as a risk factor that may increase the probability of predation.

18 Another significant risk factor arising from our survey is the presence of vegetation cover. This is in 19 agreement with previous findings by Cozza et al. (1996), who recorded a higher proportion of 20 predation attacks to livestock in presence of scrub or woodland cover compared to open terrain.

Farm and flock size, together with a high sheep/stockman ratio, also increased the risk of predation, as already recorded in another survey in Central Italy (Cozza et al., 1996), as well as in the U.S.A. (Mech et al., 2000). This can probably be explained by the fact that large flocks are more difficult to be controlled either by the stockman or by guardian dogs. Furthermore, in small farms livestock usually graze in proximity to the farmer's house, and Mech et al. (2000) recorded that the distance from human settlements increases the risk of predation, as predators usually tend to avoid humans. Larger farms also tend to have a higher sheep/stockmen ratio. Although the number of stockpersons increases with flock size, this trend is not linear, and it leads to an unfavorable ratio in very large farms, where one stockman may be in charge of more than 500 sheep. This obviously reduces the possibility of control on the animals and increases the risk of chronic predation. This may be one of the reasons why even the constant presence of a stockman with the sheep could not prevent predation, although it apparently contributed to limit sheep losses.

7 A similar problem is probably the main reason for the low effectiveness of guardian dogs. The 8 recommended sheep/dog ratio for an effective predation control is 100-150 sheep/dog (Borgia, 9 2003) or, according to Stoynov (2005), a minimum of two dogs is always required in a flock, plus 10 one dog for each 50 sheep. Our data show that the average number of sheep controlled per dog was 11 120, but this value was often exceeded, reaching peaks of 325 sheep/dog., whereas the 12 recommended sheep/dog ratio for an effective predation control is 100-150 sheep/dog (Borgia, 2003) or, according to Stoynov (2005), a minimum of two dogs is always required in a flock, plus 13 14 one dog for each 50 sheep. Moreover, it has to be taken into account that, especially in large flocks, 15 sheep are often divided into subgroups, and the number of dogs is sometimes insufficient to control 16 all these smaller groups. Another limitation to the use of guardian dogs is presence of tourists in the 17 area, as these dogs, if not well trained, can be aggressive and therefore may represent a danger for 18 people (Coppinger and Coppinger, 2005; Lüthy and Mettler, 2005). In spite of this, the presence of 19 well trained dogs has proven successful for reducing predation in other areas (Green and Woodruff, 20 1999; Marker et al., 2005; Landry et al., 2005; Berzi, 2010).

In our survey, the presence of guardian dogs was not only of low effectiveness for reducing predation, but additionally the probability for a farm to be subjected to chronic predation was even increased by the presence of dogs. Although unexpected, similar results have been previously obtained by Espuno et al. (2004) in the French Alps, and they can be explained by the fact that farmers tend to introduce guardian dogs in their flocks only after repeated attacks by predators.

1 Day and night fences apparently played no role for reducing predation. It has to be noted that, in 2 most cases, these fences had the unique function of sheep control, and, even in the case of anti-wolf 3 night fences, no electrification was present. A recent experiment carried out in the neighbouring 4 District of Florence showed a dramatic reduction of predation events following the adoption of 5 electric fences in farms severely affected by this problem (Berzi, 2010). However, in the District of 6 Pisa, the farmers are reluctant to adopt this prevention method, due to the high initial cost of 7 installation (which could be partially funded by public administration) and, above all, to the 8 following ongoing maintenance expenses.

9

10 Conclusions

11 The results of this investigation highlighted the impact of predation in the Southern District of Pisa 12 and emphasized the need for finding technical and political solutions to this problem. Attention 13 should be focused on large farms, with thick vegetation cover and located close to protected areas. 14 Although no clear indication about the effectiveness of prevention methods could be obtained from 15 our survey, studies carried out in similar areas suggest that the adoption of electric fences, well 16 trained guardian dogs and/or other prevention methods should be encouraged in-and supported by 17 the Public Administration particularly in farms with the above mentioned characteristics, with a 18 goal in order to test the effectiveness of these methods in this specific area. However, preliminary 19 information and an awareness campaign are probably necessary to encourage farmers to adopt 20 prevention methods.

21

22 Acknowledgements

This research was possible due to the initiative and support of the District of Pisa (Forestry and
Wildlife Defence Service), and we are particularly grateful to Dr Andrea Acciai, Dr Sebastiano
Boccaccio and Dr Giacomo Sanavio.

We acknowledge the collaboration of the Farmers' Association of the District of Pisa (APA), C.I.A., Coldiretti, Unione Agricoltori and to the USL 5 of Volterra, that helped us contact the farmers and gave us useful advice during this study. We are grateful to Dr Eugenio Heinzl for GIS spatial analysis and to Prof. Diane Frank for revision of the English language. Comments from an anonymous reviewer were quite useful to improve the original manuscript. Finally, we are also grateful to all the farmers who collaborated with this research.

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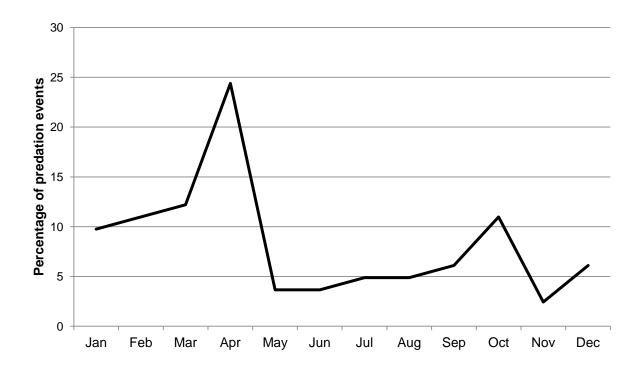
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- 20

1 Figure captions

2

Figure 1. Percent distribution of the reported predation events attributed to wolves (n=103)
throughout the year.



Distance from protected areas ¹			
≤ 5 Km	> 5 Km		
12 (26.7%)	33 (73.3%)		
17 (70.8%)	7 (29.2%)		
	≤ 5 Km 12 (26.7%)		

Table 1. Absolute (and relative) frequencies of the presence of chronic predation depending on the proximity to protected areas.

¹Minimum distance from the perimeter of the nearest protected area.

Predation	Vegetation cover			
	Null/scarce	Medium/thick		
Absent/Sporadic	21 (45.7%)	25 (54.3%)		
Chronic	4 (14.8%)	23 (85.2%)		

Table 2. Absolute (and relative) frequencies of the presence of chronic predation depending on the thickness of vegetation cover.

	Level of predation	Mean	s.e.	Min	Max	р
	Absent/Sporadic	246	38.4	10	850	0.001
Number of sheep	Chronic	497	70.7	27	1300	
Farm area (hectares)	Absent/Sporadic	73	11.3	5	300	0.01
rai in area (nectares)	Chronic	136	22.5	14	500	
Datio cham/stachuman	Absent/Sporadic	144	21.3	5	425	0.01
Ratio sheep/stockmen	Chronic	229	30.2	15	550	0.01
Runo Sheep/Stockmen	Chronic	229	30.2	15		550

Table 3. Characteristics of farm size and sheep/stockman ratio affecting the level of predation (Absent/Sporadic *vs* Chronic).

-	Use of night shelter					
Predation	Never	Occasionally	Always			
Absent/Sporadic	4 (25.0%)	4 (13.3%)	12 (44.4%)			
Chronic	12 (75.0%)	26 (86.7%)	15 (55.6%)			

Table 4. Absolute (and relative) frequencies of the presence of chronic predation depending on the use of night shelter.