

Hay Mite Communities in Sheep Farms from French and Spanish Basque Regions with regard to Scrapie Infection

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Abstract: It has been suggested that the mites connected with the decay of stored hay may be involved in the transmission of scrapie, a neurological disorder in sheep. To find out whether there are any obvious differences in the mite fauna in hay that could be related to outbreaks of scrapie in sheep, we analysed the hay fauna from 23 farms in the Basque regions in France and Spain, areas with high and very low incidence of scrapie, respectively. We did find some differences in the composition of the fauna, and a species such as *Pygmephorus* spp. was more prevalent in scrapie-infected farms, and particularly in highly scrapie-infected farms. The former species is associated with late stages in the biological degradation of stored hay and could be linked to the disease. Besides being the first record of the composition of the hay mite fauna in Spain and France our finding of differences in mite communities in hay from scrapie versus non-scrapie farms is a further indication of the possible association of certain mite species with scrapie.

INTRODUCTION

Mites in hay have been linked to outbreaks of scrapie [1]. Scrapie is the sheep equivalent to Bovine Spongiform Encephalitis (BSE). It is a slowly developing, transmissible, neurological disorder in sheep, and the causative agent is the abnormal isoform (PrP^{Sc}) of the host prion protein [2]. Outbreaks of scrapie probably occur as a result of a combination of the presence of PrP^{Sc} and the genetic susceptibility of the sheep affected. Susceptibility to scrapie is governed by polymorphisms of the PrP gene at the codons 136, 154 and 171 [3] and, in this respect, the predominant breeds in the Basque region are similar at both sides of the Franco-Spanish border [3, 4]. Scrapie is now endemic in the milking flocks of the Manech red-faced sheep breed from the French Basque region (Atlantic Pyrenees), whereas a very low incidence of the disease is reported in sheep that have nearly the same genetic constitution (the Latxa breed), in the Spanish Basque region. This suggests that as-yet unidentified environmental factors might be involved in outbreaks of the disease. During a search for possible agents of recurrent scrapie in Iceland it was found, that the mites in stored hay could be carriers of PrP^{Sc} [1, 5]. Storage mites in hay or 'haymites' have been investigated primarily in connection with human work related respiratory diseases [6, 7], but

almost nothing is known about the fauna of hay mites in most countries, including the Basque regions in France and Spain. In order to observe possible links between the species or communities of mites and the occurrence of scrapie in these otherwise genetically similar sheep breeds, we made an initial study of the mite fauna in hay in the French and Spanish Basque regions.

MATERIALS AND METHODS

Farm Characteristics

In October 2000, hay samples were collected from farms in the scrapie endemic French Basque region (8 farms with a history of scrapie -FS-, and 2 farms scrapie-free -F-) and from 7 scrapie-free farms in the Spanish Basque region (S). In order to obtain equal sized groups for statistical analysis, in October 2002 we added samples from another six French farms with no history of scrapie (F). The group characteristics of the farms can be read in Table 1. Scrapie-free status was confirmed from the absence of clinical symptoms in the flock, and the absence of signs of scrapie in culled ewes in the last years. Scrapie positive farms (FS) were classified to high (FS1, FS8, FS2, FS6) or low prevalence of the disease. High prevalence means that more than 9% of the sheep were affected, while less than 3% affected were considered to be low. These percentages were made up for each farm from maximal annual clinically confirmed cases 1998 – 2002. The distance between the farms was less than 60 km in each region.

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Table 1. Flock and Management Average Characteristics of Sheep Farms in the Basque Regions of Spain (S; Scrapie-Free) and France (F and FS; Scrapie-Free and Scrapie-Infected, Respectively)

Country	Spain	France	France
Groups	S	F	FS
Number of farms	7	8	8
Scrapie maximum incidence in % 1999-2001	0	0	10.7
% resistant sheep ARR genotypes in 1999	26	20	26
Adult ewes in the flock	234	240	241
Milk (litres per ewe)	79	138	120
Cattle per pasture area (ha)	2/ 14	18/ 25	16/ 27
Cattle-units per ha	3.0	1.9	1.7
Permanent pastures, % of grazed area	100	< 50	< 50
% Hay bought from other farms	55.1	10.4	12.6

Mite Sampling and Identification

Hay samples were collected manually and put into polyethylene bags. In most of the farms, samples were taken from both the first and second cuts, and a total of 37 samples were collected. Between 40 and 100 g of hay from these samples were processed in Berlese funnels for at least 8 hours. For identification purposes, some of the mites were stained with Lignin Pink and cleared in lactic acid before being washed with tap water on a suction filter and finally embedded in Hoyers' medium [8] on slides. They were examined under normal phase contrast x 100, and the monograph by Hughes [9] was used as guide for their identification. The remaining mites were dispersed in water in a Petri dish for counting. The mites present in an area corresponding to 10% of the dish were counted three times under a stereomicroscope at 25x magnification. The original number of mites in the sample was estimated from the arithmetic mean of these three counts, plus the number of specimens on the corresponding slide. The number of mites/kg of hay was calculated from the proportions of the various mite species identified on the slides.

Statistical Analyses of Hay Mite Communities

The following aspects of the mite communities were investigated: a) the concentrations of mite species in the hay (data transformed to $\text{Log}(x + 1)$ to stabilize the variance), b) the percentage of each species in the community (data transformed into Arcsine square root to stabilize the variance), c) the species diversity using the species number or Shannon Weaver index [10], ($H' = -\sum p_i \text{Log} p_i$ where $p_i =$ number of individuals of species i /total number of mites. H' ranged from 0 to the natural logarithm of the total number of species in the sample), and d) the presence/absence of the species, using the Farris phylogenetic Hennig86 Program [11]. The concentrations of each species of mite on the three groups of farms (F, FS and S) were compared using the analysis of variance procedures of the SAS package, version 8.0 (SAS Institute, Cary, NC, USA) with the Duncan test for

group mean comparison. The proportions of each species were compared using the same procedure.

The Hennig86 software [11, 12] has previously been used for describing communities [13] in order to trace their putative historical construction. Briefly, the original community is taken to be devoid of species (ancestral situation = outgroup) and gradually over time, it incorporates new species. Several trees were constructed, each by a single pass, but adding the farms in a different sequence each time. We then applied branch - swapping to each of the trees and finally retained just one tree corresponding to each initial tree. The findings of the analysis are presented in tree form, in which the farms are ordered on the basis of the similarity of their community. In the present paper, the "mite groups" of the farms were established from the tree (and not on the basis of their origin or scrapie status). Each cluster (mite group) of the tree based on mites is examined for scrapie, and then it is identified as low or high scrapie group. The clusters were related to scrapie, either using an analysis of variance of the mite concentrations, or the Fisher exact test for the occurrence of scrapie.

RESULTS

Number and Species of Mites

For each farm, only small and non-significant differences were found between hay from first and second cuts with regard to the mite species or the total mite counts, and so the mean value for each farm was used in subsequent analyses. The results for the single farms (Table 2) showed huge variations in the total mite concentrations, ranging from 316 to 68,112 mites per kg of dry hay.

The total number of mites per kg of dry hay did not significantly differ between the three types of farms (Table 3).

The Spanish farms had significantly higher concentrations of *Blomia freemani*, and lower concentrations of *Lepidoglyphus destructor* and "other acari", than the French farms. *Cheletomorpha lepidoptorum* and *Pygmephorus* spp. were significantly more abundant in French scrapie-infected farms and the latter was only found in these farms.

Proportions of species on the French farms (with or without scrapie) and the Spanish farms (Table 4) showed that *Acarus siro* was the dominant species in the Spanish farms accounting for 31% of the mites found; significantly lower percentages of this mite were found in the French farms. The proportion of *B. freemani* was significantly higher in Spain than in France. Conversely, the proportion of *L. destructor* was higher on the French farms, and was only found in one Spanish farm. The Shannon-Weaver index was significantly lower in the Spanish and French scrapie-free farms than in the French scrapie-infected farms. The latter were characterized by the presence of *Pygmephorus* spp.

Cladistic Analysis of Communities

Five trees were constructed, but as they were very similar to each other, only one is shown.

Most of the Spanish farms (except S1 and S3) are located in the vicinity of the outgroup, since they harboured fewer

Table 2. Number of Mites Per Kg of Dry Hay in the Scrapie-Free Sheep Farms in the Spanish Basque Region (S), in the Scrapie-Free Sheep Farms in the French Basque Region (F), and in the Scrapie-Infected Sheep Farms in the French Basque Region (FS)

Farm Code	Number of Mites Per kg of Dry Hay (Means)												
	A	B	C	D	E	F	G	H	I	J	K	L	Total
S 1	7130	0	9607	42	42	685	42	0	0	42	0	0	17590
S 2	1679	0	420	0	0	0	420	0	0	0	0	0	2519
S 3	56103	1500	3473	3564	0	1782	0	376	938	376	0	0	68112
S 4	106	0	2045	0	0	561	207	106	103	0	0	0	3128
S 5	0	0	58	0	0	467	135	0	0	0	0	0	660
S 6	0	0	0	0	0	0	402	0	471	0	0	0	873
S 7	767	0	0	0	0	153	0	0	0	0	0	0	920
F 1	106	2114	2105	0	0	0	1368	333	0	53	0	0	6079
F 2	0	183	23	121	0	8	140	4	0	0	0	12	491
F 3	0	71	15	0	107	0	71	10	0	0	0	42	316
F 4	400	2267	4800	0	2134	0	7467	933	267	133	0	799	19200
F 5	12	1061	117	0	0	0	92	25	25	0	0	43	1375
F 6	0	187	9	0	9	0	103	9	0	0	0	29	346
F 7	0	136	881	0	136	0	8000	542	68	0	0	474	10237
F 8	345	668	162	0	183	365	0	161	161	0	0	344	2389
FS 1	123	2686	437	69	0	0	137	54	260	0	191	69	4026
FS 2	172	722	352	0	0	0	181	91	344	0	172	262	2296
FS 3	177	1486	2114	441	0	0	265	0	100	0	0	0	4583
FS 4	1221	3275	5548	1710	0	0	405	243	352	162	0	162	13078
FS 5	0	1596	1482	0	2280	798	342	342	0	912	0	0	7752
FS 6	0	150	125	0	0	0	88	558	156	0	176	0	1253
FS 7	0	6607	3230	0	343	0	1284	526	722	0	0	354	13066
FS 8	67	304	1090	38	0	0	152	806	1813	0	38	134	4442

Columns: A. *Acarus siro* L. s.l.; B. *Lepidoglyphus destructor* (Schränk); C. *Tarsonemus Can.&Fanz*; D. *Tyrophagus cf putrescentiae* (Schränk); E. *Chortoglyphus arcuatus* (Tropeau); F. *Blomia freemani* Hughes; G. *Cheyletus eruditus* (Schränk); H. *Cheletomorpha lepidopterum* (Shaw); I. *Tydeus* Koch; J. *Kleemannia plumigera* Oud.; K. *Pygmephorus* Kramer; L. Other Acari; Total =Total mites per kg dry hay.

Table 3. Back-Transformed, Least-Square Means of the Concentration of Each Species of Mites (as Specimens Per Kg Dry Hay) on the Farms and ANOVA Analysis. For Each Species, Different Letters Indicate a Significant Difference in the P Value

Mite Concentrations	Spanish Scrapie-Free	French Scrapie-Free	French Scrapie-Infected	ANOVA (P Values)
<i>Acarus siro</i> s. l.	246	10	27	0.2
<i>Lepidoglyphus destructor</i>	2 a	429 b	1209 b	0.0001
<i>Tarsonemus</i> sp.	149	168	1022	0.29
<i>Tyrophagus cf putrescentiae</i>	5	1	14	0.33
<i>Chortoglyphus arcuatus</i>	1	22	5	0.2
<i>Blomia freemani</i>	90 a	2 b	2 b	0.02
<i>Cheyletus eruditus</i>	41	230	252	0.27
<i>Cheletomorpha lepidopterum</i>	69 a	66 a	133 b	0.02
<i>Tydeus</i> spp.	12	9	168	0.09
<i>Kleemannia plumigera</i>	3	3	4	0.95
<i>Pygmephorus</i> spp.	0 a	0 a	11 b	0.008
Other Acari	0 a	55 b	24 b	0.003
Total mites per kg dry hay	3377	1854	4860	0.45

Table 4. Comparisons of the Species Richness (Number of Mite Species Found on the Farm) and Shannon-Weaver (H') Index of Diversity and Percentages (Back-Transformed, Least-Square Means) of Each Taxon on the French and Spanish Farms. For Each Species, Different Letters Indicate a Significant Difference in the P Value

Farm Averages	Spanish Scrapie-Free	French Scrapie-Free	French Scrapie-Infected	ANOVA (P-Values)
Richness (No of species)	5	7	7	0.11
Shannon-Weaver index (H')	0.79 a	1.21 c	1.47 b	0.0002
% of <i>Acarus siro s. l.</i>	30.77 a	0.90 b	1.84 b	0.01
% of <i>Lepidoglyphus destructor</i>	0.04 a	30.77 b	29.12 b	0.0001
% of <i>Tarsonemus sp.</i>	14.38	10.26	23.05	0.37
% of <i>Tyrophagus cf putrescentiae</i>	0.16	0.42	1.29	0.62
% of <i>Chortoglyphus arcuatus</i>	0.00	3.57	0.84	0.16
% of <i>Blomia freemani</i>	9.72 a	0.43 b	0.17 b	0.05
% of <i>Cheyletus eruditus</i>	7.53	24.52	5.38	0.07
% of <i>Cheletomorpha lepidopterum</i>	0.14 a	3.57 b	6.36 b	0.03
% of <i>Tydeus spp.</i>	2.57	0.56	7.74	0.17
% of <i>Kleemannia plumigera</i>	0.03	0.05	0.33	0.56
% of <i>Pygmephorus spp.</i>	0.00 a	0.00 a	1.34 b	0.02

species than the French farms. They were all different and did not regroup into a cluster. The French farms harboured *Tyrophagus* spp. as part of their mite communities, and could be roughly divided into two clusters: the *Pygmephorus*-cluster (FS1, FS8, FS2, FS6 and F6), and the *Chortoglyphus arcuatus* cluster (FS4, F5, F1, FS7, F8, FS5, F4 and F7), respectively. The association of the presence of *Pygmephorus* spp. with “high scrapie” farms was highly significant (Fisher exact test; P=0.001). These findings are in agreement with the fact that the French scrapie-infected group had a significantly higher number of *Pygmephorus* spp. than the scrapie-free group (ANOVA, P<0.05). The

“high scrapie” group could be characterized as harbouring a mite community of *C. lepidopterum*, *Tyrophagus* and *Pygmephorus*.

DISCUSSION

Mite Faunistics

The dry stored hay starts a slow decomposition process after harvesting, involving a number of organisms, such as fungi, mites that graze on these fungi, and predators of these mites [14]. The idea that hay mites could be carriers of the scrapie prion protein PrP^{Sc} has its origin in Iceland [1], where

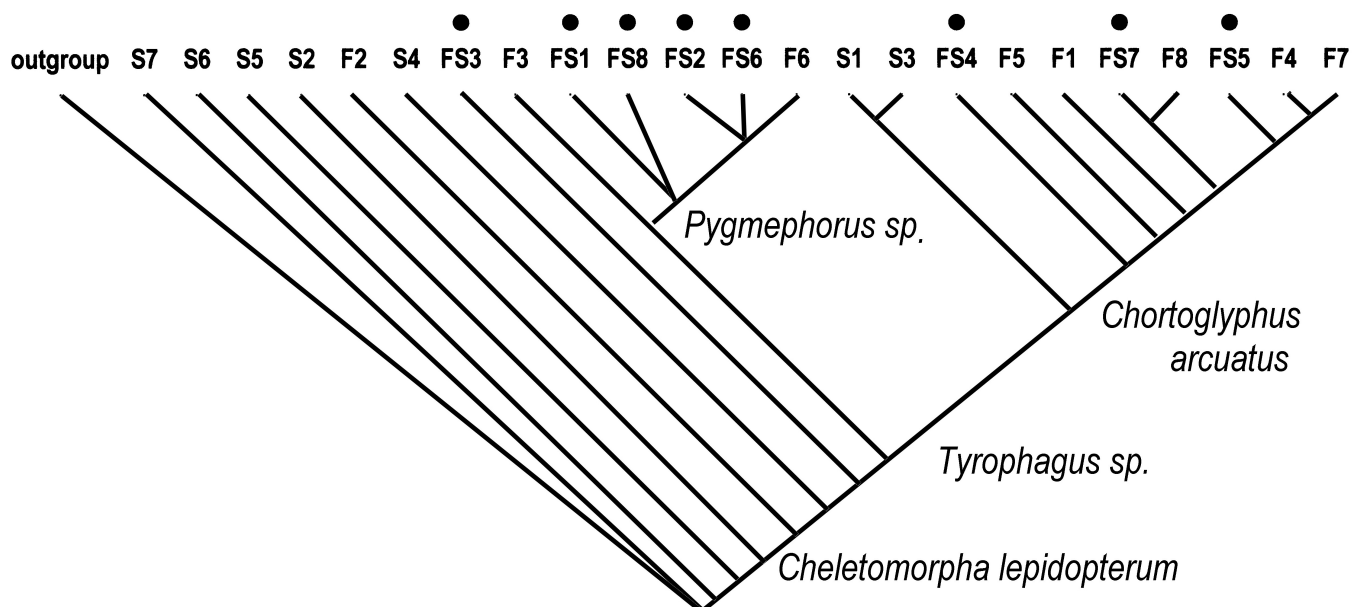


Fig. (1). Phylogenetic tree of the 23 Spanish (S) and French (F) sheep farms (*Tyrophagus* spp. cluster) based on the composition of their hay mite communities. Scrapie infected farms (FS) are identified by a black dot.

Table 5. Frequency of the Main Species of Hay Mites in Northern Europe Previously Referenced (in Brackets) and in the French and Spanish Basque Regions Studied in this Work

% of the Hay Samples in which Species was Present	Iceland [15] 1981	Faroe Islands [16] 1989	Finland [17] 1982	Denmark [18] 1990	Basque Region This Study
<i>Acarus siro s.l.</i>	81	55	64	69	61
<i>Lepidoglyphus destructor</i>	83	42	64	67	74
<i>Tarsonemus sp.</i>	97	45	55	60	91
<i>Cheyletus eruditus</i>	50	50	45	31	87
<i>Tydeus spp.</i>	78	50	82	42	61
<i>Kleemannia plumigera</i>	8	8	0	15	26
<i>Tyrophagus spp.</i>	6	40	36	54	30

the concentration of mites connected with this process is normally higher than that found in the Basque regions in this study (Table 2). A mean of 50,000 mites /kg hay was found in Icelandic farms, and concentrations of more than 1 million mites/ kg were not unusual [15]. However, hay storage conditions in Northern Europe are quite different from those in the Basque region due to different weather conditions. In Iceland, the grazing period lasts for only four months, and the farmer has to feed his flock indoors for 8 months every year. As a result, hay stores in Northern Europe are generally larger and hay is kept for longer periods (several years) than in the Basque region. However, mites also develop more slowly in Northern Europe, as the decomposition of hay is slow, due to the lower temperatures.

The frequency of the hay mite taxons found in the Basque region indicates that the hay fauna in this area included a core group of hay-inhabiting mites that is almost identical to that found in Northern Europe, including Iceland (Table 5). Quantitatively, these taxa constitute 98 - 99 % of the specimens found in the Northern European hay (i.e. from Iceland and Denmark) but they only represent 78 % of the total Basque hay mite fauna. This means, that the Basque hay fauna includes additional mite species, which are probably unable to live in Northern European hay because of the different environmental conditions, or because they are interacting with other organisms that do not occur there. Apart from the mesostigmatid *Kleemannia plumigera*, which is a fungal feeder usually associated with concrete, all the other species shown in Table 5 occupy separate niches in the mite community of stored hay [7]. The *Acarus-siro* complex *L. destructor* and *Tyrophagus* spp. are fungal feeders with chelicerate mouthparts. In the Basque samples these species seem to be replaced or accompanied by species such as *Chortoglyphus arctuatus*, *B. freemani* and *Tyrophagus* cf. *putrescentiae*. In addition, the stylet-feeding microbial grazer *Tarsonemus* sp. seems to be identical to the one found in Northern Europe, and does not appear to be replaced, but other stylet-feeder representatives of the genus *Tydeus* spp. and *Pygmephorus* spp. are larger in size than those found in Northern Europe. In the Icelandic hay another *Pygmephorus* species, *P. islandicus* Sellnick, thought to be associated with small mammals [19], was commonly found in association with biologically old hay [14]. Finally, *Cheyletus eruditus* is a stylet-feeding predator on other mites. In the Basque hay this species is accompanied by *Cheletomorpha*

lepidoptorum, which is closely related to *Cheyletus* and has almost identical mouthparts. It is thought to be associated with Lepidoptera for transport, but it is also a known mite predator [9].

There seems to be a greater variability in mite counts/kg of hay in the Spanish samples than in the French ones, although differences in the average values were not significant. This may be ascribed to the greater diversity of the sites selected in Spain, where the farms were further away from each other, whereas several of the French sites were neighbouring farms. One of the differences observed between the French and Spanish samples was the greater diversity of mites species found in France. This higher diversity was reflected in the higher Shannon Weaver index in France and was also shown on the cladogram in Fig. (1) (farms rich in mites species were distant from the species-empty outgroup). This difference could be related to the fact that the landscape is less mountainous in France than in Spain.

Mite Communities and the Presence of Scrapie

The fauna characterization in scrapie-infested farms was assessed using several statistical methods based either on raw data for the mite species, the proportion of species, or the presence or absence of specific species. *Pygmephorus* spp. was found more frequently associated and in higher proportions (raw data and proportions) in the scrapie-infested farms, which harboured a more diverse fauna. This species is characteristic of biologically old hays, which display high water activity and a low Carbon/Nitrogen ratio [14]. Phylogenetic methods indicated that the mite communities recorded in several scrapie-infested farms consisted primarily of *Cheletomorpha lepidoptorum* (a mite predator), then *Tyrophagus* spp. (a fungal feeder) and finally *Pygmephorus* spp. (microbial grazers). A few scrapie-infested farms recorded communities constituted of *Cheletomorpha lepidoptorum*, *Tyrophagus* and *Chortoglyphus arctuatus* (a fungal feeder). Not all the scrapie-infested farms were associated with the same mite communities, suggesting that half of the cases of scrapie could be linked to the presence of *Pygmephorus* spp., whereas the remaining cases would be linked to other species or environmental factors. In such a context we might expect the disease agent might be transmitted through infected food or by skin contact with a contaminated

environment with the mite acting as a carrier [20, 21]. However, the link found in this study between the presence of some hay mites and the occurrence of scrapie, may not be direct, and the mite community be only an indicator of environmental variables suitable for the promotion or persistence of the disease.

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