SHORT REPORT

Chrysotile and tremolite asbestos fibres in the lungs and parietal pleura of Corsican goats

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Background and Aims: Environmental exposures to chrysotile and tremolite from the soil cause pleural plaques and mesothelioma in northeast Corsica. Goats grazing in the contaminated areas inhale asbestos fibres. We used this natural animal model to study whether these exposures actually result in increased fibre burdens in the lungs and parietal pleura.

Methods: Ten goats from areas with asbestos outcrops and two from other areas were slaughtered. Fibre content of lung and parietal pleural samples was determined by analytical transmission electron microscopy.

Results: Both chrysotile and tremolite fibres were detected. In the exposed goats, the geometric mean concentrations of asbestos fibres longer than 1 µm were 0.27×10^6 fibres/g dry lung tissue and 1.8×10^6 fibres/g dry pleural tissue. Asbestos fibres were not detected in the lungs of the two control goats. Chrysotile fibres shorter than 5 µm were predominant in the parietal pleura. Tremolite fibres accounted for 78% and 86% of the fibres longer than 5 µm in lung and parietal pleural samples, respectively.

Conclusions: Environmental exposure in northeast Corsica results in detectable chrysotile and tremolite fibre loads in the lung and parietal pleura of adult goats. Tremolite fibres of dimensions with a high carcinogenic potency are detected in the parietal pleura.

Corsica is a Mediterranean island with a population of about 250 000 inhabitants. In northeast Corsica, a chrysotile ore body was mined between 1932 and 1965 near the village of Canari. In this area, there are also numerous deposits of serpentinites and disseminated asbestos outcrops which may cause increased levels of airborne chrysotile and tremolite fibres.^{1,2} This environmental exposure concerns about 15 000 people.³ Unlike in Turkey⁴ or Greece,⁵ there is no local use of asbestos rich soils for whitewashing or stuccoing houses. However, crushed serpentinite was used for paving roads in the area.⁶ Increased prevalences of pleural plaques and mesothelioma related to environmental exposure have been reported in northeast Corsica.^{1,3,7}

Although the parietal pleura is a common target organ for asbestos related diseases, only a few studies have determined the fibre burden in this tissue.^{8–11}

Our aim was to study the pulmonary fibre concentration which is related to environmental fibre exposure under natural conditions in Corsican goats as well as to compare the type, size, and concentration of fibres between the lung and parietal pleura in this animal model.

MATERIALS AND METHODS

Ten goats from asbestos areas in Rutali (n = 8) and Laporta (n = 2) villages in northeast Corsica were slaughtered. Their mean age was 6.4 (range 6–8) years. Samples from two unexposed goats, respectively 9 and 12 years old, from Arro village

in south Corsica were also obtained. Breeding animals were selected because their age and areas of grazing were known. Autopsy and sampling were performed after bleeding the animals to avoid blood contamination. The pleural cavities were carefully examined; no macroscopic lesions (pleural plaques, tumours, or black spots) were detected. Pieces of parietal pleura of approximately 4 cm² each were dissected from intercostal spaces in the upper, middle, and lower parts of the costovertebral region. Lung tissue samples (3–4 cm³) adjacent to the pleural samples were taken. The mean dry weights of samples analysed were 90 (25) mg for lung tissue, and 26 (16) mg for parietal pleura.

Sample preparation for lung and parietal pleural tissue and the techniques for fibre analysis by electron microscopy have been described previously.¹¹ Only structures longer than 1 µm with parallel sides and an aspect ratio (L:Ø) greater than 3:1 were considered as countable fibres. Fibre types were identified by their chemical spectrum. Fibre breakage during sample preparation affects chrysotile fibre number and dimensions to a greater extent than amphibole ones. Precautions were taken to limit its effect on measurements. Fibre concentrations are reported as fibres/gram (f/g) of dry lung or pleura. Analytical sensitivities were around 100 000 f/g dry tissue for fibres longer than 1 µm, and 50 000 f/g dry tissue for fibres longer than 5 µm.

The concentration and size of fibres (width, length, and aspect ratio) were roughly log normally distributed, thus geometric mean (GM) and geometric standard deviation (GSD) are reported. When no fibres were detected, a value of half the analytical sensitivity of the method was used for calculations.

Non-parametric Wilcoxon and Mann–Whitney U tests were used to compare fibre related variables.

RESULTS

Chrysotile and tremolite were the only asbestos fibre types detected. A few asbestos bodies, all formed on long chrysotile fibres, were detected in some lung samples.

Asbestos fibres were detected in the lungs of all the exposed goats, but in none of the controls. The concentrations did not differ significantly between the upper, middle, and lower lung samples. The highest concentration was 1.8×10^6 f/g dry lung. Mean lung tissue concentrations were below one million f/g dry lung in all exposed goats (GM (GSD) 0.27×10^6 f/g (2.8), range 0.055– 0.84×10^6 f/g).

Asbestos fibres were detected in the parietal pleura of all goats except one of the two control goats. There were no systematic differences in the fibre concentration between the different sampling sites of the parietal pleura. The topographical distribution of the concentrations was highly heterogeneous in some of the goats. For both tremolite and chrysotile the

Abbreviations: GM, geometric mean; GSD, geometric standard deviation

Table 1 Mean and range of fibre concentrations in lung and parietal pleural samples of exposed Corsican goats (in 10^6 fibres/g dry tissue)*

	Lung	Parietal pleura	p value†
Chrysotile			
Length $\geq 1 \mu\text{m}$	0.122 (3.55; 0.049 to 0.303)	1.202 (3.75; 0.467 to 3.098)	$p < 0.01$
Range	[0.015–0.477]	[0.263–17.293]	
Length $\geq 5 \mu\text{m}$	0.013 (3.04; 0.006 to 0.029)	0.011 (2.57; 0.006 to 0.022)	NS
Range	[ND–0.081]	[ND–0.055]	($p = 0.68$)
Tremolite			
Length $\geq 1 \mu\text{m}$	0.141 (2.36; 0.076 to 0.260)	0.496 (3.45; 0.205 to 1.203)	$p < 0.01$
Range	[0.029–0.459]	[0.070–3.135]	
Length $\geq 5 \mu\text{m}$	0.040 (3.28; 0.017 to 0.093)	0.077 (3.42; 0.032 to 0.186)	NS
Range	[ND–0.148]	[ND–0.419]	($p = 0.20$)

*Geometric mean (geometric standard deviation; 95% confidence interval).

†Wilcoxon matched pairs test.

ND, not detected.

mean concentrations in the parietal pleura of exposed goats were significantly higher than in the corresponding lungs ($p = 0.005$) when fibres of $\geq 1 \mu\text{m}$ in length were considered, but not when only fibres longer than $5 \mu\text{m}$ were considered (table 1; $p = 0.32$, NS). The concentrations in the parietal pleura were above one million f/g dry pleura in five exposed goats. In the exposed goats, the geometric mean concentration of asbestos fibres longer than $1 \mu\text{m}$ in parietal pleura was 1.8×10^6 fibres/g dry tissue (GSD 3.6, range 0.333 – 19.2×10^6 f/g). The pleural asbestos fibre burden did not correlate with the lung burden nor with the age of the goats. The mean pleural fibre concentration found in one unexposed goat was 0.66×10^6 fibres/g, all fibres being shorter than $5 \mu\text{m}$.

The ratio of chrysotile and tremolite concentrations was significantly higher in the parietal pleura than in the lung (3.2 ± 1.1 , Wilcoxon matched pairs test, $p = 0.016$). The chrysotile and tremolite fibres were significantly thinner and shorter in the parietal pleura than in the lung (table 2). Chrysotile fibres longer than $5 \mu\text{m}$ were found in the lungs of five and in the parietal pleura of five exposed goats; tremolite fibres longer than $5 \mu\text{m}$ were found in the lungs of nine and in the parietal pleura of nine of the exposed goats.

Ninety per cent of the chrysotile fibres in the lung and 99% in the pleura were shorter than $5 \mu\text{m}$. For tremolite fibres these

proportions were 62% and 81%, respectively (table 3). Chrysotile fibres longer than $5 \mu\text{m}$ represented 5.1% of the total lung fibre load and 1.0% of the total pleural fibre load. For tremolite fibres, the corresponding figures were 22% and 5.8%, respectively.

DISCUSSION

Two previous studies used lung fibre burden analysis in spontaneously exposed sheep to monitor environmental fibre contamination as a result of specific geological conditions in Cyprus¹² and in Cappadocia,¹³ but parietal pleura tissue was never examined.

In our study, we found evidence of increased concentrations of asbestos fibres in lung and pleural tissue samples of goats from areas with asbestos contaminated soils in northeast Corsica. These results indicate that tissue samples from local animals may be useful indicators of environmental background exposure when human samples are unavailable or scarce. Activities related to handling of the contaminated soil, such as stuccoing or whitewashing, are important determinants of the actual human exposures. Such practices are not reported in northeast Corsica. This differs from the areas with similar environmental exposure situations, which have been described in Turkey, Greece, and various other areas.^{4 12 14–16}

Table 2 Size characteristics of asbestos fibres in lung and parietal pleural samples of exposed Corsican goats

	Lung	Parietal pleura	p value*
Chrysotile			
N analysed	115	872	
Diameter (μm)			
GM (GSD; 95% CI)	0.07 (2.27; 0.06 to 0.08)	0.05 (1.90; 0.05 to 0.05)	$p < 0.001$
Range	0.02–1.0	0.01–0.5	
Length (μm)			
GM (GSD; 95% CI)	2.2 (2.00; 2.0 to 2.5)	1.5 (1.48; 1.4 to 1.5)	$p < 0.001$
Range	1.0–80.0	1.0–17.5	
Aspect ratio			
GM (GSD; 95% CI)	33 (2.65; 27 to 39)	31 (1.93; 29 to 32)	NS
Range	4–900	4–467	
Tremolite			
N analysed	108	341	
Diameter (μm)			
GM (GSD; 95% CI)	0.44 (2.33; 0.38 to 0.52)	0.31 (2.22; 0.28 to 0.33)	$p < 0.001$
Range	0.08–2.3	0.04–2.0	
Length (μm)			
GM (GSD; 95% CI)	3.9 (2.68; 3.2 to 4.7)	2.3 (1.90; 2.2 to 2.5)	$p < 0.001$
Range	1.0–50.0	1.0–17.0	
Aspect ratio			
GM (GSD; 95% CI)	9 (2.00; 8 to 10)	8 (1.69; 7 to 8)	NS
Range	3–82	3–53	

*Mann–Whitney U test.

Table 3 Proportion of chrysotile and tremolite fibres exceeding 5, 8, and 10 µm in length in lung and parietal pleural samples of exposed Corsican goats

	Lung	Parietal pleura
Chrysotile		
Length ≥ 5 µm	10.1 (19.6)	1.4 (2.0)
Length ≥ 8 µm	3.8 (5.3)	0.8 (1.7)
Length ≥ 10 µm	2.7 (4.8)	0.7 (1.7)
Tremolite		
Length ≥ 5 µm	38.0 (25.2)	18.6 (16.2)
Length ≥ 8 µm	26.2 (20.0)	7.6 (6.5)
Length ≥ 10 µm	17.2 (19.9)	3.3 (3.7)

Results expressed as mean (SD) percentage values.

We have previously reported lung fibre analysis among eight Corsican patients with environmental asbestos related disease (mean age 66 years, four with mesothelioma, one with pleural plaques, two with benign pleural effusions, and one with lung fibrosis).^{3,7} In these patients, the geometric mean concentration of fibres was 1.1×10^6 f/g dry lung for chrysotile and 7.9×10^6 f/g dry lung for tremolite. These concentrations are 8.7 and 56 times higher, respectively, than those observed in the lungs of goats, but the duration of environmental exposure was roughly 10 times shorter in the exposed goats than in the exposed patients. Thus, living nearby serpentinised rocks with asbestos outcrops in areas with a dry or Mediterranean climate, without domestic use of asbestos, may be a sufficient condition to generate abnormal lung and pleural fibre burdens.

Furthermore, the size characteristics of tremolite fibres in the lungs of the goats were close to those measured in one case of mesothelioma caused by environmental exposure in Corsica (GM (GSD): aspect ratio = 7 (1.8), length = 3.7 (2.1), diameter = 0.52 (1.9)).¹⁷ This indicates that the tremolite fibres in this case and in the goats may originate from the same source, as their size distribution is very different from that of tremolite fibres detected in lung samples of the general population and of chrysotile miners.¹⁸

The ratio of tremolite and chrysotile concentrations was higher in the above mentioned patients (7.4) than in the goats (1.2). This may be related either to differences in duration of exposure or to the faster clearance of chrysotile from the lungs. Human and animal lung fibre burden studies have consistently showed that amphiboles accumulate in the lungs to a much greater extent than chrysotile.^{19,20} Furthermore, the detection of asbestos bodies formed on chrysotile fibres in the goat lungs is probably an indicator of recent exposure.²¹

Airborne asbestos fibre measurements from exposed villages in northeast Corsica showed that both tremolite and chrysotile concentrations were higher in outdoor and indoor air samples than in control villages from northwest Corsica.⁷ However, some fibres were detected in the air samples of the control villages and this might explain the presence of short asbestos fibres found in the pleura of one of the control goats.

Only four studies including no more than 63 cases have investigated parietal pleura asbestos fibre burden in cases with pleural diseases, either pleural plaques or mesothelioma.⁸⁻¹¹ Our findings among goats are in agreement with these observations: in general, fibres in the pleura are on the average shorter than fibres in the lungs,^{8,9} most chrysotile fibres in the parietal pleura are shorter than 5 µm,⁹ and approximately 80% of the "Stanton" fibres²² are amphibole fibres.^{8,11} It remains unknown whether these differences in the size distribution and the proportions of chrysotile and amphiboles are caused by a segregation process taking place during pleural migration of fibres or whether it results from fibre breakage occurring in the pleura.

We tried to minimise the effect of heterogeneous fibre distribution in the pleura by using large samples (4 cm²) and analysing multiple samples. There was no gradient in the fibre concentrations between "upper", "middle", and "lower" parietal pleura according to the cephalocaudal axis. If fibre concentrations in the parietal pleura are influenced by anatomical differences in pleural fluid resorption, it might be more advisable to choose sampling sites along the pleural fluid pressure gradient. In goats, this pressure is highest in the sternal region.²³

In conclusion, environmental exposure to asbestos in northeastern Corsica results in increased fibre loads in the lungs and in the parietal pleura of adult goats. As with humans, the distribution of fibres in the pleura is heterogeneous. Fibres retained in the parietal pleura are shorter than those retained in the lungs. Short chrysotile fibres predominate in the pleura, but tremolite fibres of dimensions with a high carcinogenic potency are also detected.

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