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(Article begins on next page)

1 **Malignant peritoneal mesothelioma in a boar who lived in Calabria (Italy): wild animal**  
2 **as sentinel system of human health**

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24 **Abstract**

25 Mesothelioma is a tumor of the serosal membranes described both in human and veterinary  
26 medicine. While in humans the relationship between mesothelioma and exposure to  
27 asbestos and some other asbestiform minerals is well known, in animals it is still difficult to  
28 establish. In this paper a case of malignant peritoneal mesothelioma probably related to  
29 asbestos exposure in a wild boar is described. At post-mortem evaluation the peritoneum,  
30 diaphragm and serosal surface of liver and kidneys showed isolated to coalescent multiple  
31 nodular lesions. Samples from diaphragm, liver and lung were collected to perform  
32 microbiological and histological investigations. To assess the presence of asbestos and/or  
33 other asbestiform minerals, SEM-EDS investigations were performed on organs and soil  
34 samples collected from the area where the wild boar lived.

35 Microbiological investigations were negative for *Mycobacterium* species. Gross and  
36 histological examination were compatible with a biphasic mesothelioma, with nodules  
37 composed of epithelioid and sarcomatoid elements with high pleomorphism.  
38 Immunohistochemistry revealed only multifocal scattered positivity for WT-1 and D2-40.  
39 Asbestos fibres were detected in all samples (organs and soil) by SEM-EDS, demonstrating  
40 a potential relationship between the neoplasia and the exposure to naturally occurring  
41 asbestos (NOA).

42 In conclusion, the results of the present study are further confirmation that wild animals,  
43 such as the boar, are suitable sentinels to indicate the risk of environmental exposure to  
44 asbestos for human populations.

45

46

47 **Key words:** mesothelioma, asbestos environmental exposure, boar, sentinel animals

48

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50

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54

55 **1. Introduction**

56 Malignant mesothelioma (MM) is a tumor of serosal membranes including pleura,  
57 pericardium, peritoneum and tunica vaginalis testis. In humans, MM and its strong  
58 correlation with occupational or anthropogenic environmental exposure (at a high dose) to  
59 asbestos and other asbestiform minerals has been well documented and confirmed (IARC,  
60 2012) and makes it an epidemiological marker (Pavlisko and Sporn, 2014). It is more difficult  
61 to establish effects on human health resulting from chronic low-dose exposures as in the  
62 case of a natural environmental background, a very common situation.

63 The use of asbestos began in ancient times and reached the height during the second half  
64 of the 20th century. Actually mining and use of asbestos minerals remain high in a limited  
65 number of states in the world (USGS, 2018). Asbestos minerals are present in many  
66 environments, i.e. rocks, soils, air and water. Rocks are their primary source and rocks  
67 containing asbestos are widespread worldwide. Owing to natural and/or anthropogenic  
68 action asbestos is dispersed from these sources and spread in the environment.

69 The term “asbestos” covers a group of six natural minerals (hydrated silicates), one  
70 belonging to the serpentine group and the remaining five to fibrous amphiboles (IARC,  
71 2012).

72 Owing to the lack of a recognized definition, according to the dimensional definition by World  
73 Health Organization (WHO, 1997) and the literature (Belluso et al., 2017), the meaning of

74 the words used in the paper is shown below. The terms "fibre" and "fibrous" are used to  
75 address inorganic particles with a length  $\geq 5 \mu\text{m}$ , a width  $\leq 3 \mu\text{m}$ , and a length/width ratio  $\geq$   
76 3:1 which present parallel sides when seen in two dimensions, perpendicularly to fibre axis.  
77 Asbestiform is an adjective used for non-asbestos-classified fibres with asbestos-fibre-like  
78 dimensions and at least one of the asbestos properties such as flexibility or splitting. Fibre  
79 bundle is used for a parallel aggregate of mineral fibre.

80 The most widely known fibrous variety of serpentine is chrysotile which is the most  
81 commonly used asbestos in industrial production as component of asbestos containing  
82 materials (ACM). Chrysotile is abundant as naturally occurring asbestos (NOA), i. e. in rocks  
83 . Other asbestiform serpentine varieties are asbestiform antigorite and asbestiform  
84 polygonal serpentine, which are difficult to distinguish from chrysotile and often confused  
85 with it, depending on the kind of investigation (Belluso *et al.*, 2017). Other commercially  
86 common asbestos minerals include the amphiboles crocidolite and amosite (also named  
87 cummingtonite-grunerite asbestos), tremolite asbestos, actinolite asbestos and  
88 anthophyllite asbestos (NIOSH, 2011).

89 Apart from these five asbestos listed above, there are also certain asbestiform amphibole  
90 not asbestos classified as, for example, asbestiform fluoredenite (detected in Italy) and  
91 asbestiform winchite (detected in USA). Correlation between a high dose exposure to these  
92 minerals and MM was recognized through epidemiological investigations in humans affected  
93 by MM (McDonald *et al.*, 2004; Paoletti *et al.*, 2000).

94 The presence of these asbestiform minerals was identified only recently, either because  
95 they had not been detected in raw materials or because they had been confused with  
96 other minerals, such as tremolite asbestos (Meeker *et al.*, 2003).

97

98 Owing to natural and/or anthropogenic action, asbestos are dispersed from these sources  
99 and spread in various types of environments. The same origin and the same fate concern

100 other asbestiform minerals not currently classified as “asbestos” that, at the current state of  
101 knowledge, are present only in some areas of the world (Cannata et al., 2018).

102 Diffusion of fibrous minerals (whether classified as asbestos or not) in non-occupational  
103 environments has been highlighted in the last twenty years by their detection in lungs of  
104 animals. In non-experimental animals, MM is generally rare. It has been more frequently  
105 described in dogs (Forbes and Matthews, 1991; Glickman et al., 1983;) and cattle (Girard  
106 and Cécire, 1995; Klopfer et al., 1983; Zurwieden et al., 1990) but occasionally also in cats  
107 (Umphlet and Bertoy, 1988), lambs (Brown and Weaver, 1981), horses (Mair et al., 1992;  
108 Ricketts and Peace, 1976), goats (Campopiano et al., 2017) and pigs (Beytut, 2002; Uzal et  
109 al., 2016).

110 Animals have been proposed as sentinel system (SSA) for different aerial pollutants  
111 including asbestos to complement human epidemiological studies and to demonstrate the  
112 interactive effects of these mineral fibres and their role in biological response (Dumortier et  
113 al., 2002; Rey et al., 1994). In fact, animals are not affected by occupational exposure, their  
114 lungs are easier to obtain for post-mortem examination than human ones and, finally, they  
115 can provide an assessment of the kind and amount of the respirable minerals present in the  
116 environment where they lived. Therefore, SSA could be indicators of environmental  
117 background exposure to inorganic fibres (asbestos and non-asbestos) (Ardizzone et al.,  
118 2014; Capella et al., 2017a; Capella et al., 2017b; De Nardo et al., 2004; Dumortier et al.,  
119 2002; Fornero et al., 2009; Glickman et al., 1983).

120 In particular, MM in wild animals can be suitable to provide useful indications for  
121 understanding the possible correlation between low-dose exposure and carcinogenesis.  
122 Regarding the investigated Italian area (Calabria region, south of Italy), in 2017  
123 Campopiano et al. identified tremolite asbestos fibres in the pulmonary tissue of sheep,  
124 goats, and two boars that lived near disused quarries. In the Calabria region (Monte

125 Reventino, CZ) several deposits of ophiolite containing mainly tremolite both asbestiform  
126 (i.e. asbestos classified) and non-asbestiform (i.e. non asbestos classified) were reported  
127 (Zakrzewska, 2008). On the basis of the National Register of mesotheliomas in the same  
128 region 6 cases of human tumors related to asbestos exposure were detected in 2012 (INAIL,  
129 2015) confirming the presence of asbestos in the environment and the need to activate a  
130 suitable method to evaluate the potential human risk. On the basis of these considerations,  
131 the aim of this report is to describe the pathological findings of a case of peritoneal  
132 mesothelioma detected in a wild boar (from Calabria) and relate them to the environment  
133 where the boar lived.

134

## 135 **2. Material and methods**

136

### 137 **2.1 The boar**

138 A 3-year-old female wild boar of 70 Kg of weight had been killed during the hunting season  
139 in Caulonia (Reggio Calabria, Calabria, Italy) and was submitted to post-mortem inspection.  
140 The huntsman referred that the boar did not show any sign of sickness before death and it  
141 seemed in good nutritional status.

142

### 143 **2.2 Microbiological, histological and immunohistochemical investigations**

144 Samples of peritoneal nodules, diaphragm, liver, kidney and lung were collected and frozen  
145 to perform microbiological, histopathological and immunohistochemical investigations.

146

147 For microbiological investigations tissue samples were frozen and sent to the Istituto  
148 Zooprofilattico Sperimentale of Barcellona P.G. (Messina, Italy). Samples were  
149 homogenized, decontaminated with 1 volume of 4% NaOH for 30 min at 37°C, neutralized

150 with 0.067 M of phosphate buffered saline (PBS) at pH 7.2 and centrifuged for 15 min at  
151 3,000 *g*. Pellets were suspended in PBS, inoculated into Lowenstein-Jensen medium (LJ)  
152 (Biolife®, Italy) and LJ medium without glycerol (Biolife®, Italy), then incubated in CO<sub>2</sub> for  
153 8 weeks at 37 °C, according to the OIE *Manual of Diagnostic Tests and Vaccines for*  
154 *Terrestrial Animals* (World Organization for Animal Health, 2009).

155

156 After negative response for tuberculosis, frozen tissues were fixed in 10% buffered formalin,  
157 and sent to the Department of Veterinary Sciences, University of Torino for histological  
158 evaluation. Tissue samples were embedded in paraffin wax blocks, sectioned at 5 µm  
159 thickness, mounted on glass slides and stained with Haematoxylin & Eosin (HE) and  
160 Periodic Acid Schiff after diastase treatment.

161

162 Immunohistochemistry was performed on selected sections to characterize the neoplasia in  
163 an automated system (Omnis Instrument, Dako, Agilent technologies). The characteristics  
164 of the antibodies employed and the corresponding working conditions are detailed in Table  
165 1. Reactions were visualized using a polymer-conjugated secondary antibody (Envision  
166 Flex, Dako). Sections were counterstained with Mayer's hematoxylin. Positive control was  
167 represented by human tissues known to express the different markers and negative controls  
168 were carried out by omitting the primary antibodies.

169

### 170 **2.3 SEM-EDS investigation**

171 To assess the presence of asbestos and asbestiform minerals, and to evaluate a  
172 relationship between the suspected mesothelioma and the possible exposure to asbestos,  
173 the inorganic residue of lung, liver and diaphragmatic nodules samples were investigated  
174 by SEM-EDS at the Department of Earth Sciences, University of Torino following the  
175 procedure detailed in Belluso *et al.* (2006). In order to remove the organic fraction, 0.25 mg



176 of tissue from each sample have been chemically digested in 30 cc of sodium hypochlorite  
177 (NaClO). Subsequently, the solution containing the inorganic material has been filtered on  
178 a mixed cellulose ester filter with a diameter of 25 mm and porosity of 0.45  $\mu\text{m}$ . The  
179 dissolution of NaClO granules has been completed washing the filter with distilled water  
180 preheated at 60° C. The filter has been put on a SEM stub and coated with a thin carbon  
181 film.

182 Each membrane has been investigated by SEM (JEOL-IT300LV). Only inorganic particles  
183 having aspect ratio >3:1 have been considered and their dimensions have been registered.  
184 Their elemental chemical composition has been determined by EDS (Oxford Instrument  
185 INCA Energy 2000). The inorganic fibres detected were identified by comparing their EDS  
186 spectrum with those collected in the laboratory database. The distinction between chrysotile  
187 and asbestiform antigorite, that have the same chemical composition, is possible only by  
188 structural determination, but not by SEM-EDS technique (Capella et al., 2017a). As it  
189 concerns tremolite and actinolite asbestos, they only differ for a little chemical variation  
190 (Hawthorne and Oberti, 2007) that it is not possible to detect on fibres from digested tissue  
191 (Goldstein et al., 2012). Therefore, the authors grouped together chrysotile with asbestiform  
192 antigorite (i.e. chrysotile/asbestiform antigorite) and tremolite asbestos with actinolite  
193 asbestos (i.e. tremolite/actinolite asbestos).

194 The number of detected inorganic fibres has been normalized to 1 g of dry tissue (ff/gdw)  
195 as indicated by international guidelines (De Vuyst *et al.*, 1998).

196

197 Moreover, SEM-EDS investigations were performed on 5 samples of soil to assess the  
198 presence of asbestos in the environment where the wild boar lived. This area is into the  
199 woods, far away from footpaths (latitude: 38.371389; longitude: 16.390556). In the same  
200 area there are active/abandoned quarries (Italian Institute for Environmental Protection and  
201 Research -ISPRA) with some tunnels which can be used as dens by wild animals. There

202 are also some human settlement (4-5 farms) in the surrounding zone. The collection points  
203 were chosen within a circular area of about 3 km<sup>2</sup> around the site where the animal was  
204 felled, considered it as its home range (Figure 1). On the basis of these considerations, more  
205 or less equidistant points of about 500 meters were identified from the killing point of the  
206 animal. For each site, approximately 1 kg of soil to a depth of 30 cm was collected after  
207 elimination of the layer of foliage and shrubs typical of the subsoil.

208 A representative portion of each soil sample was processed following the guidelines of the  
209 Italian legislation (Italian Ministerial Decree of September 6, 1994). From the dried fraction  
210 with diameter < 2 mm, a portion of soil was ground by using an agate pestle and mortar and  
211 5 mg of obtained powder was suspended into a dispersive solution. A share of this solution  
212 was filtered on a polycarbonate filter with a diameter of 25 mm and a porosity of 0.8 µm. The  
213 filter was put on a SEM stub and coated with a thin carbon film. The presence of inorganic  
214 fibres was evaluated on 0.1 mg of soil by SEM-EDS. Their amount was calculated to obtain  
215 the value of mg/kg (ppm) as indicated by the Italian legislation (Italian Ministerial Decree of  
216 September 6, 1994; the asbestos pollution being with value equal to or more than 100  
217 mg/kg).

218

### 219 **3. Results**

220

#### 221 **3.1 Post-mortem evaluation**

222 At post-mortem evaluation the peritoneum, surface of diaphragm and serosal surface of liver  
223 and kidneys showed isolated to coalescent multiple nodular lesions varying from 2 mm to 3  
224 cm in diameter from grey-white to red-yellow color depending on the amount of  
225 hemorrhages. The nodules had a smooth and translucent surface (Figure 2). On the serosal  
226 surface of the liver some nodules were pedunculated with a cauliflower like surface due to

227 many complex fissures. No lesions were found in the thoracic cavity. On the basis of the  
228 observed lesions tuberculosis/mesothelioma was suspected.

229

### 230 **3.2 Microbiological, histological and immunohistochemical investigations**

231 The microbiological exam resulted negative for *Mycobacterium* spp., excluding the  
232 hypothesis of Tuberculosis.

233

234 The histological features of the nodules were the same in all the samples (diaphragm, kidney  
235 and liver serosal surface) and hepatic and renal parenchyma were not affected. Moreover,  
236 in liver hemosiderin accumulations were observed probably suggesting previous  
237 hemorrhages. Lungs showed no histological alterations.

238 Nodules were composed by a mixed cell population of epithelioid and sarcomatoid elements  
239 with an high pleomorphism. The epithelioid cell population showed oval cells with abundant  
240 and light-colored/eosinophilic cytoplasm, round-to-oval nuclei and well-defined cell borders  
241 (Figure 3). These cells did not show reactivity with PAS stain indicating the absence of mucin  
242 droplets within tumor cells or tubular lamina which were characteristic of adenocarcinoma  
243 (Head, 1990). Less commonly binucleate or multinucleate cells were observed. The  
244 sarcomatoid pattern was composed by pleomorphic spindled fibroblast-like cells. Areas of  
245 necrosis and lymphoplasmacytic infiltrates with rare neutrophils were frequently observed.  
246 Both cell populations were mixed in the nodules and tended to dispose in papillary structure,  
247 nests or cords.

248

249 Most of the immunohistochemical investigations failed to help in the classification of the  
250 tumor masses. In fact, only multifocal scattered WT-1 and D2-40 positivity were detected  
251 (Figure 4). All the samples showed negativity for Calretinin, cytokeratin 5, HBME-1 and CEA,

252 but at least for the former three no positivity in internal control cells was observed, thus the  
253 reactions were considered inconclusive.

254 Localization and morphological pattern are compatible with the diagnosis of a biphasic  
255 mesothelioma containing both epithelioid and sarcomatoid components at histological  
256 examination. Malignancy was supported by the presence of necrosis, increased cellularity,  
257 pleomorphism and a diffuse proliferation in the connective stromal tissue supporting  
258 mesothelial cells.

259

### 260 **3.3 SEM-EDS investigation**

261 The inorganic fibres detected in different organs at SEM-EDS investigation are reported in  
262 Table 2. In lung, mineral fibres attributable to phyllosilicates and amphiboles families, have  
263 been detected, both asbestos and asbestiform minerals. Within the asbestos minerals,  
264 tremolite/actinolite asbestos (Figure 5–a,b), and chrysotile/asbestiform antigorite (Figure 5-  
265 c,d) were detected. Regarding asbestiform minerals, hornblende, Na-Ca amphiboles and  
266 illite-smectite have been detected.

267 In the diaphragmatic nodules and in the liver only chrysotile/antigorite asbestiform fibres  
268 have been detected.

269 Inorganic fibres detected in soils samples are reported in Table 3. Chrysotile/asbestiform  
270 antigorite is present only in 2/5 samples while fibrous amphibole species (both asbestos and  
271 non-asbestos classified) have not been detected.

272 Two kinds of fibrous species, chrysotile/asbestiform antigorite and fibrous illite/smectite have  
273 been detected both in biological and soil samples. In both types of samples, the amount of  
274 chrysotile/asbestiform antigorite is predominant compared to the other fibrous minerals.

275

## 276 **4. Discussion**

277 Primary peritoneal tumors in animals are rare. One of this is represented by peritoneal  
278 mesothelioma even if its diagnosis must be made with caution because of the similarity of  
279 mesothelial proliferative lesions with chronic granulomatous peritonitis and metastatic  
280 tumors in serous membranes (such as carcinoma) (Head, 1990).

281 In the present case Tuberculosis was suspected during post-mortem inspection as the  
282 peritoneum was covered by diffuse small nodules. However, histological examination  
283 revealed no granulomatous inflammatory process and microbiological investigations  
284 excluded the suspect of mycobacterial infection.

285 Gross and histological examination are compatible with a biphasic mesothelioma as the  
286 nodules were composed by both epithelioid and sarcomatoid mixed components (Head,  
287 1990). Moreover, immunohistochemistry, although focally, was positive for WT1 e D2-40  
288 which are specific markers for mesothelioma (Ordoñez, 2013) supporting the hypothesis of  
289 a MM in the boar. Indeed, other tested mesothelioma-specific markers, such as calretinin,  
290 cytokeratin 5 and HBME-1, were negative both in tumor cells and in internal control cells  
291 (such as normal peritumoral mesothelial cells). This finding appears difficult to explain but  
292 the authors assume that it can be due to bad antigen preservation in tissue samples. All the  
293 samples have been frozen before chemical fixation and the type of freezing-defrosting  
294 process (temperature, delay in tissue allocation) can influence the antigen preservation  
295 (Pelstring, 1991).

296 In human medicine mesothelioma usually develops in the pleural cavity (Bianchi and  
297 Bianchi, 2014). On the contrary, in veterinary medicine a higher number of cases of  
298 peritoneal mesothelioma have been reported in literature (Bacci *et al.*; 2006; Beytut, 2002;  
299 Brown and Weaver, 1981; Forbes and Matthews, 1991; Girard and Cécire, 1995; Umphlet  
300 and Bertoy, 1988). This different localization could be explained by the capability of  
301 pulmonary macrophages with phagocytosed dust particles to move through alveolar walls  
302 towards the pleura. In doing so they may penetrate bronchioles and be transported by the

303 mucociliary staircase or they may penetrate blood vessels and be carried to extrapulmonary  
304 sites where they excite a reaction for example in the liver, spleen, kidney and abdominal  
305 wall (Holt, 1981). In the boar of the present case an ingestion of asbestos fibres cannot be  
306 excluded.

307 The evidence of abundant asbestos fibres in lung, liver and diaphragmatic nodules with  
308 SEM-EDS methods seems to support this pathological mechanism and the diagnosis of  
309 mesothelioma relating to asbestos exposure.

310 The mineral fibres detected in the biological samples are compatible with the geological  
311 characteristics of the area where the wild boar lived. In particular chrysotile/asbestiform  
312 antigorite was detected both in biological and in soil samples. The authors cannot exclude  
313 that the lack of detection of fibrous amphiboles in soil samples is due to a detection limit of  
314 SEM-EDS method.

315 To the best of author knowledge, in humans tremolite asbestos accumulates in lung, while  
316 chrysotile is quickly cleared. This difference in pulmonary clearance can explain why  
317 tremolite asbestos and amphiboles asbestos in general are considered "2-3 orders of  
318 magnitude more carcinogenic than chrysotile" (WHO, 2015). Assuming that pulmonary  
319 clearance rate in wild boars is similar to human one, the authors can hypothesize that  
320 probably this animal respired more chrysotile/asbestiform antigorite than tremolite/actinolite  
321 asbestos, but the most persistent fibres are left the most abundant.

322 Moreover, the amount of fibres detected in the boar lungs is lower than that reported in  
323 previous studies.

324 As it concerns the amount of fibres in the lung sample (Table 2), the comparison with  
325 literature data shows that amphibole asbestos is much lower than that found in lungs of  
326 sheep, goats and two boars from more than 100 km far from the provenance site of the  
327 examined boar (Campopiano *et al.*, 2017). Similarly, goats from Corsica (Dumortier *et al.*,  
328 2002), cows from north-western Italy (Belluso *et al.*, 2017), cats and dogs from California

329 (Abraham *et al.*, 2005) showed lower amount of amphiboles fibres in lung compared to the  
330 boar of the present study. Also for chrysotile/asbestos antigorite, the burden in cow lungs  
331 from north-western Italy is lower (Belluso *et al.*, 2006) than that found in the investigated  
332 boar. These animals did not develop MM even if they had a higher fibre burden compared  
333 to the boar.

334 In humans a burden of amphiboles asbestos (fibre with length of  $> 5 \mu\text{m}$ ) higher than  $1 \times$   
335  $10^5/\text{gdw}$  is considered an indicator of significant asbestos exposure (De Vuyst *et al.*, 1998),  
336 able to trigger cancerous pathologies. It is interesting to note that the amphiboles asbestos  
337 burden in the boar is even lower than the threshold for humans.

338 This finding, correlated with the mesothelioma diagnosis, seems to support the hypothesis  
339 of a cancer risk related to natural environmental exposure (low doses) to asbestos, and the  
340 presence of a genetic component of susceptibility to MM (Crovella *et al.*, 2016) also in  
341 animals.

342 For these reasons the role of wild boars as SSA has to be considered central in: i)  
343 determining the environmental diffusion of asbestos and non-asbestos classified  
344 asbestiform minerals; ii) establishing if the natural exposure to asbestos could play a role in  
345 the development of human mesotheliomas.

346

## 347 **5. Conclusion**

348 In conclusion, the results of this study suggest that an active surveillance on regularly  
349 slaughtered domestic/wild animals seems suitable to quantify the risk of exposure to  
350 asbestos for human population.

351 It would be interesting to carry out diagnostic investigations also on MM cases in other  
352 wild/domestic animals to confirm the hypothesis that, at least in animals, the low-dose  
353 asbestos respiration, presumably continuous, and for a limited period of time (3 years) may  
354 induce MM.

355

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536 **Figures Legends**

537 **Figure 1.** Geographical area where the boar was killed and soil samples were collected.

538

539 **Figure 2.** Boar, macroscopical findings at post-mortem examination. A: peritoneum,  
540 diaphragm and serosal surface of abdominal organs covered by multiple nodules. B: detail  
541 of the caudal abdomen characterized by diffuse serosal proliferations.

542

543 **Figure 3.** Boar, histological characterization of the nodular lesions. A: proliferations attached  
544 to the diaphragm, Haematoxilin and eosin stain (H-E). B: mixed cell population composed  
545 by epithelioid and sarcomatoid elements with disseminated areas of necrosis and  
546 lymphoplasmacytic infiltrates. H-E, 100x. C: detail of the tumor masses attached to the  
547 serosal surface characterized by pleomorphism and nest or cords organization. H-E, 200x.

548

549 **Figure 4.** Boar, immunohistochemical characterization of the nodular lesions. A: multifocal  
550 scattered WT1 positivity. Immunohistochemistry for WT1 detection, haematoxylin  
551 counterstaining, 400x. B: multifocal D2-40 positivity. Immunohistochemistry for D2-40  
552 detection, haematoxylin counterstaining, 400x.

553

554 **Figure 5.** SEM images (2000X) and relative EDS spectra of chrysotile/asbestiform antigorite  
555 (a,b) and tremolite/actinolite asbestos (c,d) detected in lung samples of the boar.

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558 **Table legends**

559 **Table 1.** Immunohistochemical protocols.

560 **Table 2.** Inorganic fibres detected in different kinds of tissue (ff/ gdt: number of fibres per  
561 gram of dry tissue; \* fibre bundle of 2 fibres minimum; \*\* fibre bundle of 6 fibres minimum)

562 **Table 3.** Inorganic fibres detected in soil samples of 5 different areas (\* fibre bundle)

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