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# Mesothelioma in Domestic Animals: Cytological and Anatomopathological Aspects

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## 1. Introduction

Normal mesothelial cells form a monolayer that covers the serosal surface of pleural, peritoneal and pericardial cavities. When these cells are injured by inflammation or altered in a neoplastic process, they acquire similar cytological characteristics that difficult differentiation between reactive mesothelial cells and neoplastic ones (Ogilvie & Moore, 2008).

Mesotheliomas emerge from the cells that cover serosal cavities (Head et al., 2002). They are really uncommon neoplasms in animals and they are usually malignant (Head et al., 2003). They are more frequent in bovine cattle and dogs, but they represent just 0,2 % of the total of canine neoplasms (Wilson & Dungworth, 2002). There are exceptional reports in horses (Hinrichs et al., 1997), cats, pigs and other species (Brown et al., 2007).

In human beings the relationship between these tumours and asbestos fibre is striking (Butnor et al., 2001; Cantin et al., 1982; Enzinger & Weiss, 1985). In veterinary the presence of asbestos bodies was just established in a few canine reports (Glickman et al., 1983; Harbison & Godlesky, 1983) but controversy exists since many authors consider there is no convincing association between the incidence of mesothelioma and exposure to asbestos (López, 2007). Many other fibres types may cause mesotheliomas probably owing to fibre size and solubility (Brown et al., 2007). It is also related with the exposure to simian virus 40 (SV40) (Reggetti et al., 2005). Lately, there is emerging evidence of genetic variation in susceptibility to fibre carcinogenesis (Rud, 2010).

Congenital mesotheliomas are described more frequently in bovine fetuses and young animals (Brown et al., 2007). In canine specie, the average age of onset is 8 years, but there are reports in puppies (Glickman et al., 1983; Kim & Choi, 2002; Leisewitz & Nesbit, 1992). Other authors consider that these tumours are more common in male and spayed bitches, while there does not seem to be any strong breed predilection (Head et al., 2002). Exposure to pesticide is a cofactor that apparently increases the risk of mesothelioma in the animals (Glickman et al., 1983).

The "Histological classification of tumours of the serosal surfaces (pleura, pericardium, peritoneum, and tunica vaginalis) of domestic animals" (Head et al., 2003) considers the

existence of benign and malignant mesotheliomas. It describes three varieties for each one: predominantly epithelioid, predominantly fibrous and biphasic or mixed. In human beings, half of malignant mesotheliomas are epithelioid, 20% are fibrous and the remaining 30 % are mixed (De May, 1996). Epithelioid and biphasic mesotheliomas are the most frequent types in dogs (Trigo et al., 1981), horses and cats (Head et al., 2003). Benign tumours are uncommon, many pathologist think that all the mesotheliomas are potentially malignant as virtually all can spread by implantation rather than metastasis (Brown et al., 2007).

## 2. Anatomopathological aspects

Mesothelial tumours are local, multifocal or diffuse neoplasms arising from the mesothelial lining of coelomic cavities and consisting of a variable mixture of epithelioid and spindle – shaped cells. (Head et al, 2003). They may involve one of the cavities or all of them simultaneously. Bovine lesions are usually epithelioid and primary in the peritoneum, with spread to other coelomic cavities. In dogs, these tumours involve the pleura, peritoneum, pericardium or tunica vaginalis in decreasing order of frequency. Pleural presentation is typical in swine (Brown et. al., 2007). In animals, pleural location usually causes effusion with resulting respiratory distress, cough, and weight loss (López, 2007). The peritoneal form is usually associated with ascitis as a consequence of blockage and effusion of lymphatic's vessels (Enzinger & Weiss, 1985). In these cases peritoneal fluid may appear bloody or milky (Brown et al., 2007).

They usually appear as multiple firm, sessile, or arborescent nodules, from a few milimeters to 6, to 10 cm in diameter: in villus projections on a thickened mesentery or serosal surface (Figure 1); or as fibrous or sclerosing forms, which are more plaque- like (Brown et al., 2007).



Fig. 1. Macroscopical aspect of a peritoneal predominantly epithelioid malignant mesothelioma in a dog.

3. Histopathological and cytological aspects

Usually mesotheliomas in animals, appear as a solid mass made up of layers of dark, plump cuboidal, columnar or rounded, epithelioid cells with a distinct border and abundant pink cytoplasm, over proliferating fibrocellular stroma. Mitotic figures are typically not numerous. The mesothelial cells form loops and festoons in a papillary pattern, or line cystic spaces and tubular structures (Brown et al., 2007). Asbestos fibres generally form big structures covered by acid mucopolysaccharides known as “ferruginous bodies” but they are not always seen in histologic samples (Enzinger & Weiss, 1985). Mesotheliomas can be either benign or malignant. However, usually only the malignant varieties are associated with effusions (De May, 1996). Malignant mesotheliomas frequently start causing effusion, but cytological diagnosis may be difficult because malignant and reactive mesothelial cells are morphologically very similar. During inflammation, mesothelial cells become reactive and not only increase in number, but also become pleomorphic and form multinucleated cells that may be mistaken for a carcinoma (López, 2007).

Three different histological types of these tumors are described (Head et al., 2003). This classification is presented in table 1. The epithelioid malignant and mixed types are most likely to result in an effusion containing diagnostic cells. Predominantly fibrous mesotheliomas seldom cause an effusion and when they do, rarely exfoliate diagnostic cells (Tao, 1989). Therefore, the diagnosis of mesotheliomas is based in two different cytological techniques. The analyses of effusions` sediment is recommended for epithelioid malignant mesothelioma, but for predominantly fibrous and mixed forms, fine needle aspiration is necessary (Koss et al., 1988).

<i>Benign mesothelioma</i>
Predominantly epithelioid benign mesothelioma
Predominantly fibrous (spindle cell) benign mesothelioma
Biphasic (mixed) benign mesothelioma
<i>Malignant mesothelioma</i>
Predominantly epithelioid malignant mesothelioma
Predominantly fibrous (spindle cell) malignant mesothelioma
Biphasic (mixed) malignant mesothelioma

Table 1. Histological Classification of Tumours of Serosal Surfaces of Domestic Animals: Tumours of Mesothelium. Head et al., 2003.

3.1 Benign mesothelioma

Benign presentation is rare. Some pathologists think all mesotheliomas are potentially malignant.

3.1.1 Predominantly epithelioid benign mesothelioma

This benign tumor is mainly composed of mesothelial cells resembling epithelium arranged in a papillary, tubular, or solid pattern, either alone or in combination.

When these tumours grow on the surface of serosal membrane, they show papillary branching tree- like outgrowths with a central stromal core. But when the epithelioid cells



extend into the underlying stroma in a tubular pattern, microscopically gives a pseudoacinar appearance. Sometimes they grow in solid pattern forming trabeculae or cords (Head et al., 2003).

The fine needle aspiration biopsy specimen is abundantly cellular. Sheets of reactive or atypical mesothelial cells, with windows, are seen in the aspirate specimen (Tao, 1989). Mesothelial cells are cuboidal or polygonal and have distinct outlines, a few may show a hair like brush border. The cytoplasm exhibits vacuoles sometimes of glycogen (Head et al., 2003). The nuclei are central, round to oval and relatively regular and uniform. The chromatin is fine and there may be small, inconspicuous nucleoli (De May, 1996).

They are difficult to differentiate from a reactive mesothelium, and from well differentiated malignant mesotheliomas because cells have similar appearance. So, differential diagnosis may require clinical and other complementary diagnostic techniques correlation (De May, 1996).

### **3.1.2 Predominantly fibrous (spindle cell) benign mesothelioma**

This is the less common variant of mesotheliomas in animals. It is mainly composed of spindle-shaped mesothelial cells with elongated nuclei, sometimes forming a whorled pattern, resembling a fibroma (Head et al., 2003).

Fine needle samples show variable cellularity, usually scarce and may be bloody. The cells are spindle shaped and resemble fibroblasts with a poorly defined cytoplasm. The nuclei are small, oval to elongated, and have fine chromatin and inconspicuous nucleoli (Dusenbery et al., 1992). Naked nuclei may be numerous. Metachromatic stromal fragments are frequently present. No mitoses are seen (De May, 1996).

### **3.1.3 Biphasic (mixed) benign mesothelioma**

This type may show a mixture of variable proportions of epithelial and spindle cells. In such cases, the epithelial component may be reactive while the spindle component is neoplastic (De May, 1996).

## **3.2 Malignant mesothelioma**

Malignant and benign mesotheliomas have a similar basic structure, so that in some cases it can be difficult to establish a diagnosis. The presence of neoplastic cells in lymph vessels at some distance from the deep surface of the tumours and proven lymph node metastases are distinct proof of malignancy. Tumor cells with marked anisocytosis and anisokariosis, arranged in solid masses. Mitosis may be more frequent, but some metastatic mesotheliomas have few mitotic figures. (Head et al., 2003). Usually mesothelial cells show a clear perinuclear region with variable amounts of vacuoles. Sometimes, these vacuoles' fusion leads to formation of mucin lakes producing cellular cohesion loss (Figure 2) (Enzinger & Weiss, 1985). Areas of necrosis may undergo dystrophic mineralization, especially in cattle (Head et al., 2003).

These tumours are frequently associated with a milky or blood-tinged effusion. So, fluid cytology is usually the first diagnostic study performed. Most are exudates (De May, 1996). Effusions are usually present as the result of blocked lymphatics (Brown et al., 2007). The

fluid is characteristically viscous to gelatinous, which is primarily due to hyaluronic acid. It could be present in benign diseases but high levels of hyaluronic acid ( $>8$  mg/dl) are more specific for mesothelioma (De May, 1996).

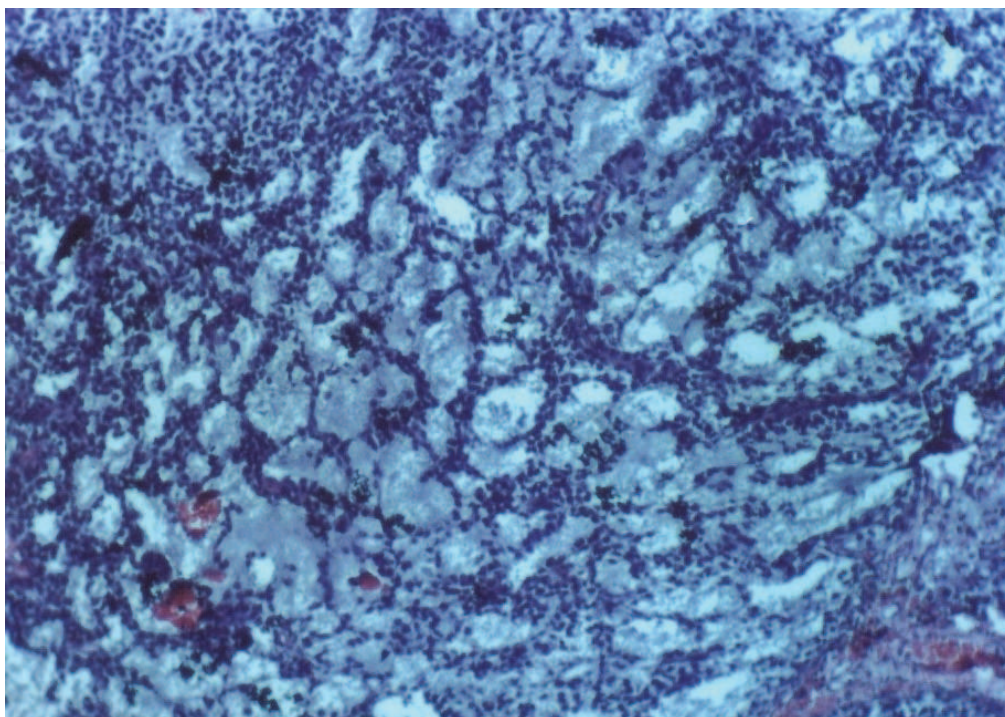


Fig. 2. Predominantly epithelioid mesothelioma. Mesothelial cells forming trabeculae or cords with abundant extracellular mucin lakes. H y E, 100 X.

Fine needle biopsy smears are usually very cellular with abundant papillae, biphasic combination of epithelioid and spindle cells and clinical findings help the diagnosis (Sterret et al., 1987).

### 3.2.1 Predominantly epithelioid malignant mesothelioma

Is the most common type, not only in humans but also in animals. It is composed of epithelioid mesothelial cells with varying degrees of anaplasia and invasive growth into the underlying tissue (Figure 3), lymphatics and blood vessels (Head et al., 2003).

Cytological samples show neoplastic cells resembling normal mesothelial cells, they may appear in big groups or isolated. There is usually a continuum from bland to malignant-appearing cells, rather than a separate, discrete population of malignant cells, as is seen with metastatic tumor. Occasionally, all the malignant mesothelial cells have only a bland, reactive appearance, that, difficult the diagnosis of malignancy. On the other hand, many cases are composed of anaplastic cells, in which the diagnosis of malignancy is obvious, but the cell of origin is not (De May, 1996).

The cells can form cohesive flat sheets with prominent windows, three-dimensional cell balls or papillae, or tubular/acinar-like structures (Jayaram et al., 1988). Cells in groups are more numerous, bigger and tend to be more irregularly arranged in malignant mesothelioma than in benign proliferations. Cells borders are prominent (Baker & Lumsden,

2000). Thick clusters of cells with highly irregular or knobby outlines are characteristic of mesothelioma (Figure 4). This is important in differential diagnosis from adenocarcinomas since the last one tend to form cell clusters with smooth continuous borders. In some cases, the cells are poorly cohesive, with numerous single cells. Papillary clusters reflect the growth pattern of mesothelioma, so, they are less common in adenocarcinoma and benign effusions. Another common feature in malignant mesotheliomas is long chains of cells known as “cell-embracing”, “cell engulfment” and “cannibalism” (De May, 1996).

The cells shape can vary from round to polygonal to angular. A minor component of spindle cells is common (Reuter et al., 1983). The cytoplasm is abundant with relatively well defined cell borders. In Diff Quick, the cells may have ruffled cytoplasmic borders corresponding to microvilli, ultrastructurally (Craig et al., 1992). The cytoplasm varies from dense an squamoid (remiscent of immature squamous metaplasia, as seen in the Pap smear) to delicate and vacuolated or foamy. Vacuolated signet ring-like cells may be present. The vacuoles may be degenerative in nature or contain metachromatic, mesenchymal mucin (hyaluronic acid). Some cells contain lipid. The presence of vacuolated, epithelioid cells may suggest adenocarcinoma (Koss et al., 1988).

The nuclei can be centrally or eccentrically located. They are round to oval, and can vary from uniform to pleomorphic (Reuter et al., 1983). Binucleation or multinucleation is common (Cowell et al., 1991). The chromatin ranges from fine to coarse depending on the differentiation. Nucleoli, angular, single or multiple may be prominent (Baker & Lumsden, 2000). Mitotic figures occur, but are not useful in distinguishing benign from malignant mesothelial cells, unless frankly abnormal (De May, 1996). Although psammoma bodies and asbestos bodies can be seen (Tao, 1989), neither is specific for malignant mesothelioma, or even for malignancy.

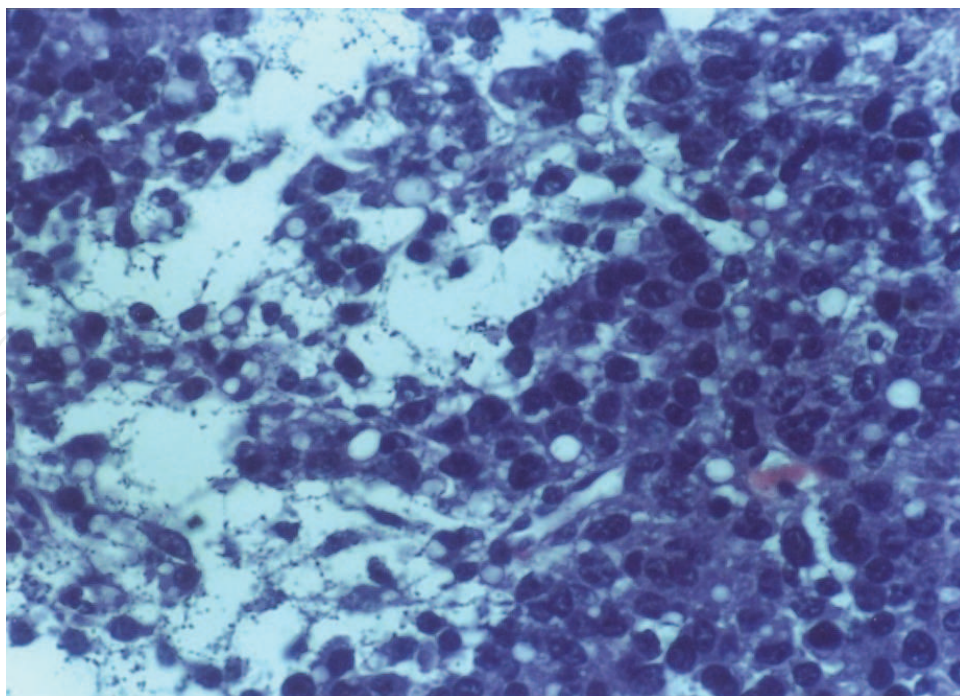


Fig. 3. Neoplastic mesothelial cells proliferation in a solid pattern, with nuclear pleomorphism, coarse chromatin, multiple cytoplasmic vacuoles and extracellular mucus. H y E., 400X



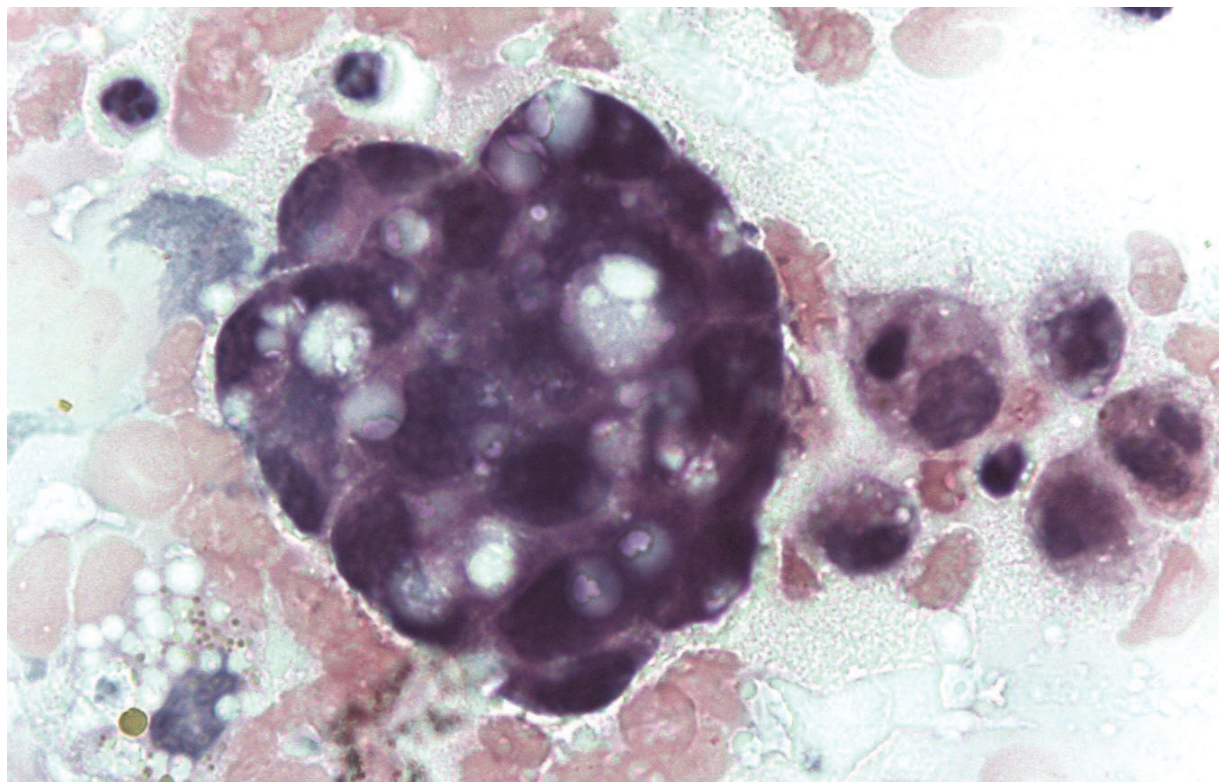


Fig. 4. Effusion sediment. Cluster of malignant mesothelial cells with typical knobby borders. H y E, 1000X.

### 3.2.2 Predominantly fibrous (spindle cell) malignant mesothelioma

A malignant mesothelial tumor resembling a fibrosarcoma and showing infiltration into the underlying connective tissue (Head et al. 2003). This type is generally localized, rarely cause effusions and its sediment usually shows low cellularity (Enzinger & Weiss, 1985; Tao, 1989). So, fine needle biopsies may be particularly important in the diagnosis of this variety of mesothelioma. These specimens have variable amounts of cells depending on fibrosis degree. The cells may be single or in loose clusters, sometimes forming whorls and storiform patterns. The cells are spindle shaped and may have long cytoplasmic processes. The cells have a moderate amount of cytoplasm that varies from delicate to well defined. The nuclei are relatively large, oval and variably pleomorphic, often with coarse, hyperchromatic chromatin (De May, 1996). Nucleoli are small, but prominent, and frequently multiple. Naked nuclei may be conspicuous (Tao, 1989). Mitoses and necrosis are common (Enzinger & Weiss, 1985). Rarely osseous or cartilaginous metaplasia may occur (De May, 1996).

### 3.2.3 Biphasic (mixed) malignant mesothelioma

Is rather frequent. Usually the epithelioid pattern predominates in the specimens but that may vary in different areas and cases (De May, 1996).

## 4. Differential diagnosis

The primary consideration in the differential diagnosis is adenocarcinoma, particularly of the lung, but intestinal and genital metastasis must be also discarded (Head et al., 2003).



Whether, mesothelioma or adenocarcinoma, any malignant tumour with extensive pleural spread is essentially incurable. However, the diagnosis of mesothelioma may be crucial from a medicolegal point of view in humans (De May, 1996).

Special stains, electron microscopy and immunocytochemistry may be helpful in differential diagnosis. Both, mesotheliomas and adenocarcinomas produce mucinous substances that may be differentiated by means of special stains.

Neoplastic mesothelial cells may produce even intra or extracellular acid mucin, hyaluronic acid which have mesenchymal origin (Di Bonito et al., 1993), whereas, adenocarcinomatous cells may secrete neutral mucins (epithelial origin). Mesotheliomas very rarely take up neutral mucin stains like mucicarmine and PAS diastase, but they usually have intracellular positive vacuoles to alcian blue (De May, 1996). Acid mucin also stains metachromatically with toluidine blue (Enzinger & Weiss, 1985).

Electron microscopy shows long, slender, abundant, microvilli in mesothelioma, they usually have a length-to-diameter ratio in excess of 12 to 1 (Head et al., 2003) while in adenocarcinoma, microvilli are short and stubby (De May, 1996). The cytoplasm contains numerous bundles of tonofilaments arranged circumferentially around the nucleus (Wilson & Dungworth, 2002).

Immunohistochemistry is also useful in differentiating these tumors since mesothelial cells uniquely express both epithelial cytokeratins and mesenchymal markers such as vimentin (Mc Donough et al., 1992). Mesotheliomas and adenocarcinoma are positive for low molecular weight keratin, while just the former is positive for high-molecular-weight keratin. They are also positive to calretinin, N-cadherin (Abutailly et al., 2002), desmin and P-cadherin (Merlo et al., 2007), but they are negative for carcinoembryonic antigen (CEA) and Leu-M1 (CD 15) (De May, 1996).

There is a report of a peritoneal mesothelioma in a dog of unusual morphologic variant of epithelial mesothelioma, with remarkable cytomorphologic resemblance to decidua. In this case, immunohistochemistry showed strong, diffuse, cytoplasmic staining of neoplastic cells for pancytokeratin and cytokeratin AE1/AE3 and focal, cytoplasmic staining in scattered cells for cytokeratin 5/6. Tumor cells also stained intensely positive for vimentin, whereas anticalretinin, smooth-muscle actin, desmin, S-100 protein and CD117 were negative (Morini et al., 2006).

There is also a description of a lipid rich pleural mesothelioma in a dog, which immunohistochemically, expresses both cytokeratin and vimentin markers as is expected in a mesothelioma. But it also shows S-100 expression, what is consistently found in liposarcoma (Avakian et al., 2008).

## 5. Conclusion

Mesotheliomas are extremely rare diseases in animals. The relationship between these tumours and exposure to asbestos and other fibres with similar size and solubility is accepted in human beings but in animals there are not many documented proves about this association. Different authors describe congenital mesotheliomas in bovine foetuses and many others consider they are more common in male dogs and spayed bitches.

The histological classification considers benign and malignant mesotheliomas with three different varieties: predominantly epithelioid, predominantly fibrous and biphasic or mixed, but the first one is the commonest. Malignant forms usually cause effusions, so, cytological analysis of the sediment is in general the first approach to diagnosis.

Its differentiation from other entities may be so difficult and may comprise since reactive mesothelial cells responses till adenocarcinomas. So, many complementary diagnostic tools may be required for an accurate diagnosis. Most of histopathological and cytological descriptions are based in human mesotheliomas but its striking features are found in animals neoplasms. Lately, many veterinary cases have been reported, based in different immunohistochemical analyses aimed at proving mesothelial cells origin.

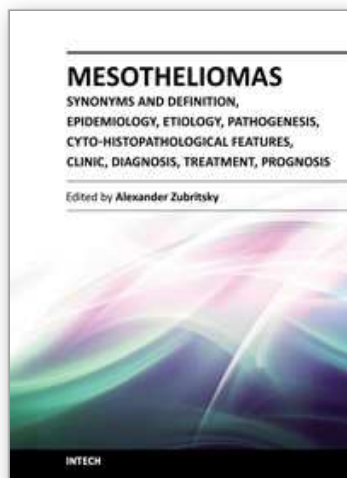
Even though, “whether, mesothelioma or adenocarcinoma, any malignant tumor with extensive serosal spread is essentially incurable” (De May, 1996) and animal illnesses usually don’t have medicolegal implications, an accurate diagnosis is also expected in veterinary science.

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**Mesotheliomas - Synonyms and Definition, Epidemiology, Etiology, Pathogenesis, Cyto-Histopathological Features, Clinic, Diagnosis, Treatment, Prognosis**

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Mesotheliomas are mysterious mesothelial tumors in that they are relatively rare, difficult to diagnose, with a large number of synonyms, and the etiology and pathogenesis of the disease are still not fully disclosed. This problem attracts the attention of various specialists in the field of medicine and biology every year. In recent years there has been a significant increase of mesothelioma morbidity in most of the countries, due to the further industrialization of society. In this regard, this book has been published with the participation of an international group of experts with rich experience from around the world . The book consists of 14 chapters containing the most advanced achievements of all aspects of the various types of mesotheliomas, both in humans and domestic animals, at a high methodological level. This book is intended for biologists and all health care workers, mostly oncologists of different profiles, as well as students of medical educational institutions engaged or even just interested in the problems of mesotheliomas.

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