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What is This?
Blood and Serous Cysts in the Atrioventricular Valves of the Bovine Heart

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Abstract. A survey of 30,907 slaughterhouse cattle (5,984 calves, 15,937 young adult, 8,986 cows) was carried out to determine the incidence of blood and serous cysts on atrioventricular valves. The cysts were classified by their content (blood/serous fluid), location (mitral/tricuspid valve), and size. Cyst wall samples were processed for histology, immunohistochemistry for factor VIII-related antigen, and transmission electron microscopy. The content of some cysts was studied by electrophoresis and biochemical and microbiologic methods. Older cows had a higher incidence (16.2%) than younger animals (11.5% in calves, 7.9% in steers, 6.4% in heifers), suggesting that the lesions may be acquired. Blood cysts were often present on both atrioventricular valves; serous cysts prevailed on the mitral valve. Cysts of both types were larger in older animals; serous cysts were larger than the blood cysts. Histologically, blood cysts contained fresh blood, and serous cysts were filled with a hyaline fluid devoid of cells, sterile, and biochemically similar to lymph. All the cysts were lined with endothelium, but a positive immunostaining for the factor VIII-related antigen was appreciable only in blood cysts. Ultrastructurally, the endothelium was composed of flat endothelial cells holding several cytoplasmic filaments, lying in blood cysts on a continuous and often laminated basal lamina with many cytoplasmic projections. The results support the hypothesis that cysts of the atrioventricular valves derive from the dilation of blood and lymphatic valvular vessels, do not regress with age, and are mainly the result of mechanical effects.

Key words: Atrioventricular valves; blood cysts; cattle; endothelial cells; factor VIII-related antigen; heart; immunohistochemistry; ultrastructure; valvular cysts.

Material and Methods

Hearts from 30,907 cattle slaughtered from June to October 1991 in a private abattoir near Bologna, Italy, were examined to investigate the prevalence of valvular cysts. The animals examined were 5,984 calves (4-5 months of age, Holstein-Friesian males), 15,937 young adults (14-15 months of age, Holstein-Friesian and Limousine, 14,024 steers and 1,913 heifers), and 8,986 cows (5-8 years of age, Holstein-Friesian).

The hearts were longitudinally sectioned, and the atrioventricular valves were carefully examined for the presence of cysts, which were classified according to their content as blood or serous cysts. When both types of cysts were present in the same heart, the prevailing type only was scored. In a series of 412 affected hearts, the valvular cysts were counted and measured and their anatomical location (tricuspid or mitral valve) was recorded.

For the histologic examination, representative samples from affected valves (20 blood cysts and 20 serous cysts for each age class) were fixed in 10% buffered formalin and embedded in paraffin. Five-micrometer-thick serial sections were stained with hematoxylin and eosin, Orcein-Van Gieson’s stain for elastic and collagen fibers, Gomori’s stain for reticulin, and
Table 1. Prevalence of cysts of the atrioventricular valves in 30,907 cattle.

<table>
<thead>
<tr>
<th>Age Class</th>
<th>n</th>
<th>No. Cysts</th>
<th>Prevalence (%)</th>
<th>Blood Cysts</th>
<th>Serous Cysts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Calves</td>
<td>5,984</td>
<td>687</td>
<td>11.48</td>
<td>408</td>
<td>6.82</td>
</tr>
<tr>
<td>Young adults</td>
<td>15,937</td>
<td>1,227</td>
<td>7.70</td>
<td>544</td>
<td>3.41</td>
</tr>
<tr>
<td>Steers</td>
<td>14,024</td>
<td>1,104</td>
<td>7.87</td>
<td>489</td>
<td>3.49</td>
</tr>
<tr>
<td>Heifers</td>
<td>1,913</td>
<td>123</td>
<td>6.43</td>
<td>55</td>
<td>2.88</td>
</tr>
<tr>
<td>Cows</td>
<td>8,986</td>
<td>1,454</td>
<td>16.18</td>
<td>624</td>
<td>6.94</td>
</tr>
<tr>
<td>Total</td>
<td>30,907</td>
<td>3,368</td>
<td>10.90</td>
<td>1,576</td>
<td>5.10</td>
</tr>
</tbody>
</table>

Perls' stain for hemosiderin and with the periodic acid–Schiff (PAS) reaction.

For immunohistochemistry, sections from 20 specimens (10 blood cysts and 10 serous cysts) were dewaxed and rehydrated, and endogenous peroxidase was blocked with 3% hydrogen peroxide for 60 minutes at room temperature (RT). The sections were then incubated with Protease XIV (Sigma, St. Louis, MO) 0.05% in Tris buffer for 5 minutes at 37 C. After preincubating for 30 minutes with 1:20 normal goat serum (Dakopatts, Copenhagen, Denmark), the sections were allowed to react with the primary antibody, rabbit anti-human von Willebrand factor (Dakopatts) diluted 1:3,000 overnight at 4 C. Biotinylated goat anti-rabbit immunoglobulin (BIO SPA, Milan, Italy; diluted 1:100) followed by peroxidase streptavidin (BIO SPA, diluted 1:250) were used as secondary and tertiary staining reagents, respectively, at RT for 30 minutes. Diaminobenzidine (Sigma) (0.04%, 7 minutes) was used as chromogen, and the sections were then counterstained with Papanicolaou hematoxylin.

For transmission electron microscopy, valvular cysts samples (three blood cysts and three serous cysts for each age class) were excised from the hearts about 30 minutes after slaughter. Specimens 1-3 mm thick were fixed for 3 hours in 5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2, rinsed in the same buffer, and postfixed for 1 hour in 1% osmium tetroxide in 0.1 M cacodylate buffer, pH 7.2. After dehydration in a graded sequence of alcohols, the specimens were then embedded in Durcupan AcM resin. Semithin sections were stained for light microscopy with toluidine blue, and ultrathin sections were stained with uranyl acetate and lead citrate and examined in a Philips CM10 transmission electron microscope.

Protein concentration of the serous cyst content and of the blood serum from two calves, two steers, one heifer, and two cows was quantified using a commercial assay (Bio Rad, Richmond, CA), and protein analysis was performed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis as previously described. Gels were stained using Coomassie brilliant blue R-250 and analyzed by densitometric scanning (Model 620 Densitometer, Bio Rad). Molecular weights of single bands were established by comparison with prestained low-molecular-weight markers (Bio Rad).

Chloride concentrations of the same samples of blood serum and serous cyst content were determined by a Stat Profile 4 Analyser (Nova Biomedical, Waltham, MA).

For microbiologic examination, 0.01 ml from cyst fluid samples (nine blood cysts and nine serous cysts, three samples for each age class) were cultured on the following solid media: blood agar, Gassner medium, brain-heart infusion, Yoder agar, and Protease-3-agar and on the following fluid media: Yoder broth, SAT medium, and tyoglycolate broth. Cultures were incubated at 37 C in normal atmosphere and in an atmosphere containing 12% CO₂. Solid media were incubated for 96 hours and examined at 24-hour intervals. Liquid media were examined every 24 hours, and after 48 hours of incubation 0.01-ml samples were transferred to solid media and incubated as indicated above.

Results

The valvular cysts prevalence in the 30,907 examined cattle is listed in Table 1.

Macroscopic findings

Macroscopically, the cysts were always sessile and roundish or oval and protruding above the atrial surface of the valvular leaflets (Figs. 1, 2). They were 1 mm to 3 cm in diameter. The serous cysts were significantly larger than the blood cysts by analyses of variance (ANOVA; P < 0.001), and the cysts on the mitral valve were larger than those on the tricuspid (P < 0.001). Both types of cysts showed a tendency to enlarge with advancing age (Table 2).

Fig. 1. Mitral valve, heart; cow. A large blood cyst (arrow) (diameter = 1.5 cm) and a serous cyst (arrowhead) (diameter = 1.0 cm) are close to the valve base of the two valvular leaflets.
Blood cysts were mostly unilocular and frequently multiple (Table 3) (up to seven on the same valve). In some cases, several blood cysts were grouped together so that the lesion had a multilocular appearance.

Serous cysts were unilocular and usually single (Table 3). When the same valve exhibited more than one serous cyst (maximum of three), these were generally located on separate leaflets. The serous cysts were generally close to the base of the valvular leaflet (Fig. 2), but the largest ones showed a tendency to invade the whole surface of the leaflet.

Blood cysts were most frequently present on both mitral and tricuspid valves except in cows, where they prevailed on the tricuspid valve; serous cysts were preferably located on the mitral valve in all three age classes (Table 4). A serous cyst was also found on a chorda tendinae of a papillary muscle of the mitral valve in one cow, and a second cyst arose from the endocardium of the left ventricular wall in another cow.

Blood and serous cysts were often associated in the same valve (Fig. 1), and in some hearts the coexistence of blood and serous cysts was observed in both AV valves.

Table 2. Diameter (mm) of blood and serous cysts of the atrioventricular valves in 412 cattle.

<table>
<thead>
<tr>
<th>Age Class</th>
<th>Mitral Valve</th>
<th>Tricuspid Valve</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Blood Cysts</td>
<td>Serous Cysts</td>
</tr>
<tr>
<td></td>
<td>Mean Range</td>
<td>P</td>
</tr>
<tr>
<td>Calves*</td>
<td>79 2.7 1–10</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Steers/heifers†</td>
<td>140 3.5 1–15</td>
<td>NS†</td>
</tr>
<tr>
<td>Cows§</td>
<td>193 4.0 1–20</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* P values for ANOVA of calves versus steers and heifers.
† P values for ANOVA of steers and heifers versus cows.
‡ NS — not significant.
§ P value for ANOVA of calves versus cows.
Table 3. Number of cysts present on the atrioventricular valve leaflets of 412 cattle.

<table>
<thead>
<tr>
<th>Age Class</th>
<th>Mitral Valve</th>
<th></th>
<th>Tricuspid Valve</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Blood Cysts</td>
<td>Serous Cysts</td>
<td>Blood Cysts</td>
<td>Serous Cysts</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>Range</td>
<td>Mean</td>
<td>Range</td>
</tr>
<tr>
<td>Calves</td>
<td>79</td>
<td>2.1</td>
<td>1–6</td>
<td>1.4</td>
</tr>
<tr>
<td>Steers and heifers</td>
<td>140</td>
<td>1.5</td>
<td>1–4</td>
<td>1.2</td>
</tr>
<tr>
<td>Cows</td>
<td>193</td>
<td>1.3</td>
<td>1–3</td>
<td>1.2</td>
</tr>
</tbody>
</table>

Ultrastructural findings

Blood cysts were internally lined by flat endothelial cells having ovoid or convoluted nuclei with irregular borders and scarce cytoplasm containing several filaments, mitochondria, ribosomes, and micropinocytotic vesicles (Fig. 3). The cell boundaries showed numerous cytoplasmic projections that were fingerlike at the luminal face and rootlike at the abluminal face. The endothelial cells lay on a continuous basal lamina with frequent aspects of duplication and lamination. The wall of the blood cysts was composed of elastic and collagen fibrils and fibroblasts in an amorphous matrix containing proteoglycan particles.

The endothelial cells lining serous cysts (Fig. 4) were similar to those of the blood cysts, although their cytoplasmic filaments were far more numerous and arranged parallel to the basal lamina or encircling the nucleus. Adjacent endothelial cells were closely connected by extensive overlapping of fingerlike cytoplasmic projections, and rootlike processes projecting into the underlying tissue were rare. The basal lamina was single layered or slightly duplicated, but it never showed any lamination as seen in blood cysts. The serous cyst wall was composed of loose connective tissue rich in proteoglycans, with fibroblasts and scattered elastic and rare collagen fibrils.

Other investigations

The results of the biochemical and electrophoretic analyses of the serous cyst fluid and blood serum are shown in Table 5.

Microbiologic tests were negative for both types of cysts.

Discussion

Hematocysts of the AV valves, up to 1 cm in diameter and sometimes multiple, are considered common in calves, especially newborns. Previous reports have stated that these cysts do not usually persist for more than a few months, except in rare cases in which they may enlarge and persist for more than 1 year, during which time their content changes into a serous fluid.6,20

In one study,2 cysts of the AV valves, up to pea-size and filled with blood or serous-water fluid, were said to affect up to 75% of calves and to regress during the life time by resorption and organization phenomena.

The results of our study contrast these widespread ideas: 1) blood cysts and serous cysts may be regarded as separate entities; and 2) cysts of the AV valves do not regress with age, and their incidence and dimension are significantly increased in older animals.

Blood cysts affected both valves, were small, and contained fresh blood. Their endothelium was immunohistochemically positive for the factor VIII-related antigen, and the wide lamination of the basal lamina was as described in blood vessels endothelia,5 both factors indicative of their blood vessel origin.17 The observed focal discontinuities of the immunopositivity may be the result of the dilation of the larger cysts; as previously noted,17 the positivity for this antigen becomes weaker as the caliber of vessels increases.

Serous cysts were larger and usually solitary and more frequently affected the mitral valve. The lack of endothelial reaction to factor VIII-related antigen, the extensive overlapping of the fingerlike projections of adjacent cells, and the numerous perinuclear filaments in the endothelial cells are regarded as distinctive features of lymph vessels.13,17

Electrophoresis and biochemical analyses showed an analogy of components between serous cyst fluid and blood serum. Nevertheless, the cyst fluid contained a significantly ($P = 0.0002$) lower concentration of total

Table 4. Anatomical location of the valvular cysts of 412 cattle.

<table>
<thead>
<tr>
<th>Age Class</th>
<th>Blood Cysts</th>
<th>Serous Cysts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tricuspid</td>
<td>Both</td>
</tr>
<tr>
<td>Calves</td>
<td>79</td>
<td>15 (21%)</td>
</tr>
<tr>
<td>Steers and heifers</td>
<td>140</td>
<td>37 (33%)</td>
</tr>
<tr>
<td>Cows</td>
<td>193</td>
<td>63 (46%)</td>
</tr>
</tbody>
</table>
Fig. 3. Transmission electron micrograph. Mitral valve, heart; steer. The endothelial cells (E) lining the blood cyst lie on a thick and multilayered basal lamina (BL), pierced by rootlike cytoplasmic projections (arrows). Uranyl acetate–lead citrate. Bar = 1 μm.

proteins, with different proportions of albumin and globulin, and a slightly \( P = 0.1 \) higher amount of chloride. These differences indicate that the serous cyst content is similar to lymph.\(^{16,21}\)

Several hypotheses have been formulated to explain the mechanism of valvular blood cyst formation in humans: 1) hematomas, 2) angiomas, 3) ventricular endothelial infoldings of the valve leaflet bulged into the atrium from the pressure gradient during the valve closure and producing valvular diverticula, and 4) ectasia or dilation of blood vessels.

Cyst morphology excludes a traumatic or neoplastic genesis. The third hypothesis assumes the existence of connections between the cyst lumina and the ventricles via small endothelium-lined channels,\(^{26}\) but these connections have not been observed in this or in other\(^{24}\) studies.

Our results support the fourth hypothesis, as recently proposed for canine blood valvular cysts,\(^{24}\) and suggest that bovine blood and serous cysts of the AV valves may originate from the dilation of blood and lymph vessels, respectively. Cysts of the AV valves morphologically equivalent to these bovine serous cysts have been observed in slaughtered pigs and were empirically regarded as distended lymphatic vessels.\(^{8,10,18}\)

The existence of blood and lymphatic vessels in the AV valves was at one time controversial\(^{1,7}\) but has been definitely proven in both humans and animals.\(^{12}\) In cattle, a particularly profuse valvular capillary plexus, which extends to the closing edges of the AV valves, has been described\(^{23}\) and may be considered a predisposing factor for the high incidence of valvular cysts in this species. Furthermore, a ventricular subendocardial network of small blood and lymph vessels that drain into branches ascending the papillary muscles and chordae tendinae has been described in the hearts of pigs, dogs, sheep, cattle, and humans.\(^{5,23}\) The two atypical serous cysts observed in a chorda tendinae and in the left ventricular wall of two cows in this study were located along the course of lymph vessels.

The higher frequency of larger valvular cysts in cows than in young cattle contrasts with the general opinion.
that valvular cysts are congenital lesions that regress during the first year of life. Our observations support the hypothesis that valvular cysts persist and enlarge during the entire life time and that they may also be acquired later in life. Likewise, low-degree congenital ectasias of vessels might become grossly observable only after age-related enlargement.

Etiologic factors leading to blood cyst formation are unknown. It has been supposed that mechanical effects, such as an increase in tension, friction, and impact, can trigger the enlargement of valvular blood vessels in a sort of valvular telangiectasis with subsequent cyst formation.24

Sex does not appear to play any role in the development of valvular cysts. The fact that all the affected calves were male and all the adults were cows was only a sampling artifact. The group of young adults, formed by male and female cattle, showed a similar incidence of cysts in both sexes.

In addition to the progressive enlargement of the valvular cysts, advancing age seems to correlate with a decrease in the number of blood cysts and an increase

### Table 5. Electrophoretic and biochemical analysis of the fluid contained in the serous cysts and of the homologous blood serum.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total protein (g/dl)</th>
<th>Albumin (g/dl)</th>
<th>Immunoglobulin (g/dl)</th>
<th>Hemoglobin (g/dl)</th>
<th>Chloride (mEq/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Range</td>
<td>Mean</td>
<td>Range</td>
<td>Mean</td>
</tr>
<tr>
<td>Serous cyst content</td>
<td>2.9</td>
<td>2.2-4.3</td>
<td>1.35</td>
<td>0.85-2.9</td>
<td>0.06</td>
</tr>
<tr>
<td>Blood serum</td>
<td>6.0</td>
<td>5.1-7.0</td>
<td>1.77</td>
<td>1.09-2.38</td>
<td>0.36</td>
</tr>
<tr>
<td><em>p</em></td>
<td>0.002</td>
<td>0.08</td>
<td>0.0009</td>
<td>0.02</td>
<td>0.1</td>
</tr>
</tbody>
</table>

* t-test for paired samples.
Specific bovine predisposition:
- profuse valvular capillary plexus
- ossification of the anulus fibrosus

Age predisposition:
- progressive obstruction and diminution of lymphatics
- loosening of the spongiosa

in the number of serous cysts. A change from blood to serous content does not seem likely, however, because blood and serous cysts have a different predilection for mitral and tricuspid valves and a different anatomical structure. Moreover, in this large sample of animals no form of transition between blood and serous cysts was observed.

The predilection of the serous cysts for the mitral valve could be related to a lower capacity of the lymphatics, which have thinner walls than hematic vessels, to withstand the dilation consequent from the systolic pressure gradient, which is stronger in the left ventricle than in the right one. This explanation may also suffice for the larger dimension of the cysts located on the mitral valve.

At the atrial surface of the tricuspid and mitral valves, there are lymph capillaries that extend from the free margins of the cusps to the anulus fibrosus of each valve, where they drain into a channel passing through the myocardium and joining the subepicardial duct of the atrioventricular sulcus. The bovine physiologic age-related ossification of the anulus fibrosus may hinder the lymph outflow through the transmyocardial channels and facilitate the dilation of the valvular lymph vessels, which may thus explain the high frequency and large dimensions of serous cysts in adult cattle.

Other age-related predisposing factors (Fig. 5) would be the progressive obstruction and diminution of the valvular blood and lymph vessels and the progressive loosening of the spongiosa stratum of the AV valves.

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