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BOVINE PANCREOLITHIASIS IN DENMARK

By

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VELLING, KNUD: *Bovine pancreolithiasis in Denmark*. Acta vet. scand. 1975, 16, 327—340. — The results of a study of the occurrence of bovine pancreolithiasis in Denmark are given and discussed. Among 65,471 heads of cattle slaughtered at 9 export slaughter-houses during the fourth quarter of 1969, 279 cases were found, giving an average frequency of 0.43 %. The frequency among animals below 4 years of age was 0.19 %, above 4 years of age 0.82 %. A difference in frequency according to breed was demonstrated. It was highest in the Red Danish breed, lower in the Holstein-Friesian breed and lowest in the Jersey breed. Clinical symptoms related to the condition were not found in a retrospective analysis, and correlations with other pathological changes were not apparent. Geographically the cases were uniformly distributed.

At 1 export slaughter-house the collection of cases was continued also in the 3 first quarters of 1970. On a 1 year basis comprising 142 cases a higher frequency was found in the late autumn and a lower during the summer.

Patho-morphological changes in the affected glands were examined macro- and microscopically. The white, hard, faceted stones were located in the excretory duct system with an accumulation in the main duct, from where a certain expulsion to the duodenum occurs. Secondary inflammatory changes were found in the epithelium and outer layers of the duct system, whereas no significant changes were found in the incretory or excretory components of the gland.

The highest absolute and relative weight of the collected and dried stones from each individual case was found in the 3—5 years age group.

Chemical analyses showed the main constituent to be calcium carbonate in the modification of calcite, and small amounts of phosphate and magnesium. A comparative chemical analysis on pancreatic stones from cattle in France, England and Denmark did not show any significant differences.

pancreolithiasis; bovine; Denmark; epidemiology; pathology; chemistry.

Since the first report on a case of bovine pancreolithiasis was published by *Furstenberg* (1846) in Germany, the occurrence of this condition up to 1967 was only known from sporadic

casuistic reports from different nations. Earlier Scandinavian reports are limited to 2 casuistic records by *Sahlsted* (1919) comprising 1 case and *Dalgaard* (1945) comprising 3 cases.

In 1967—69 an intensive research on the topic was made in France, initiated by „L'Institut National de la Santé et de la Recherche Médicale“ and performed at „Le Laboratoire de l'Unité de Recherches Chirurgicales M₄, Pavillon M, Hôpital Edouard-Herriot“ in Lyon. Preliminary results of the research were published by *Vérine et al.* (1968) and *Vérine* (1968). *Charvet* (1969) compiled the literature, comprising a total of 53 cases which were supplemented with 47 cases collected in France from 1967 to 1969, and discussed all available data at that time.

Prompted by an invitation from l'Institut National de la Santé et de la Recherche Médicale to participate in an international research on the topic a parallel Danish examination of the condition was performed in the same period.

Preliminary reports comprising some of the results were published by *Velling & Vérine* (1971) and *Vérine* (1971). The present paper gives a survey and discusses the results of the Danish study.

MATERIAL AND METHODS

Veterinary inspectors at 9 public slaughter-houses and export slaughter-houses were asked to collect all cases of pancreolithiasis found among slaughtered cattle during the period of October 1 through December 31, 1969.

Geographically the slaughter plants were chosen throughout the country (Ålborg, Ålestrup, Holstebro, Herning, Randers, Odense, Slagelse, Nakskov and Rønne) with the aim to examine a theoretically possible difference in the incidence under different soil conditions.

In 1 slaughter-house (Odense), the examination continued a full year in order to examine a possible seasonal variation.

Technically the meat inspectors were asked to perform a thorough palpation of the eviscerated pancreas (together with the intestines), a procedure not normally required. In case of a positive finding of stones the whole organ was dissected free from the intestines. Pieces of the gland were immediately cut out and fixed in 10 % neutral, buffered formalin in 100-ml plastic jars. The fresh and fixed glands were sent to the depart-

ment week by week, followed by written information concerning the owner (if available) of the animal, the breed, sex and age, and pathologic conditions found during the routinely performed inspection procedures of each individual case.

Information about the age of all slaughtered cattle during the examination period was also obtained. This could be given only as below or above 4 years of age (without or with complete eruption of permanent teeth) except for the Odense material, which permitted a division in below or above 2 years of age.

In the cases where it was possible to obtain the owner's name and address a follow-up-questionnaire was submitted, asking for precise information regarding breed, sex and age of the animal; whether veterinary service had been required during the last month before slaughter and if so, for which ailment, and finally the reason why the animal had been sold for slaughter.

In the department the glands were examined grossly and weighed. All major pancreatic ducts were opened, the stones were washed in a jar, dried on a 50°C hot plate and weighed. A few of the glands were X-ray-examined prior to further processing.

The fixed tissues were routinely processed. Paraffin-embedded 4 μ sections were stained with Iron-hematoxylin (F. C. C. Hansen) — v. Gieson and v. Kossa.

Chemical analysis of the dried stones included qualitative and quantitative methods. X-ray diffraction analysis was performed on 6 samples of stones, chosen at random*. Calcium, magnesium and phosphate analyses were performed on 20 samples of stones, also chosen at random. One g of stones was boiled with 50 ml of 2 N-HCl until disappearance of all soluble material (15—30 min.). After cooling the unclear solution was diluted up to 250 ml and filtered. Calcium and magnesium were determined on the filtrate by atomic absorption photometry. Phosphate was determined by spectrophotometry on reduced phosphomolybdate. Calcium was further determined by EDTA-titration on 2 samples. Carbon dioxide determination on further 2 samples was performed according to the method of Scheibler**.

* Performed at the Department of Inorganic Chemistry, Royal Veterinary and Agricultural University, Copenhagen.

** Performed at the Laboratory of Agricultural Chemistry, Royal Veterinary and Agricultural University, Copenhagen.

RESULTS

Anamnestic information

A total of 65,471 heads of cattle were slaughtered and examined during the 3 months period. Two-hundred-and-seventy-nine cases of pancreolithiasis were found, giving an average frequency of 0.43 %.

The group below 4 years of age consisted of 41,401 heads of cattle, of which 80 or 0.19 % had developed pancreolithiasis. Among the 24,070 animals above 4 years of age 199 cases or 0.82 % were found (Table 1).

Table 1. Number of cases of pancreolithiasis.

| Age | Number of examined animals | Pancreolithiasis | |
|-----------|----------------------------|------------------|------|
| | | number of cases | % |
| < 4 years | 41,401 | 80 | 0.19 |
| > 4 years | 24,070 | 199 | 0.82 |

The total number of slaughtered animals below 4 years of age consisted of approx. 10—15 % females and 85—90 % males, whereas the animals above 4 years of age consisted of approx. 95 % females and 5 % males.

Pertinent anamnestic information was obtained from the owners in 131 cases. Among these, 4 breeds were represented: Red Danish breed (RDM), Holstein-Friesian (SDM), Jersey breed (Jersey) and cross-breeds. The distribution between breeds was compared with the general distribution of the breeds in the nation, based on figures obtained from official statistics. Because of considerable fluctuations in the general breed distribution during the years, the average distribution between 1960 and 1966 was calculated, harmonizing the age distribution in the general population and the pancreolithiasis cases. The frequency of bovine pancreolithiasis seems somewhat higher in RDM than expected, in SDM somewhat lower and in the Jersey breed considerably lower (Table 2). One-hundred-and-one cases were in female and 30 in male animals.

During the last month before slaughter veterinary assistance was asked for in 3 of the cases, and in each of these because of mastitis. The reasons for delivery to slaughter were generally

Table 2. Distribution according to breed related to the general distribution of the breeds in the nation.

| | 1960 % | 1966 % | Average of 1960 and 1966 % | % cases of pancreolithiasis |
|--------------|-----------|-----------|----------------------------------|--------------------------------|
| RDM | 61.1 | 47.8 | 54.5 | 67.7 |
| SDM | 18.1 | 29.3 | 23.7 | 9.7 |
| Jersey | 15.1 | 16.4 | 15.8 | 2.9 |
| Cross-breeds | 4.5 | 5.7 | 5.1 | 2.5 |

Figures from 1960 and 1966 according to official statistics 1970.

because of cessation of lactation from 1 or more quarters due to mastitis, old age or too low yield.

Other findings at the meat inspection did not reveal any correlation with pancreolithiasis and other generally occurring pathologic changes.

The geographical distribution of the cases of pancreolithiasis showed a uniform distribution among the examined areas.

Table 3. Seasonal distribution of pancreolithiasis in examined animals below and above 2 years of age (Odense).

| | Number of ex- amined animals < 2 years | Pancreoli- thiasis | | Number of ex- amined animals > 2 years | Pancreoli- thiasis | |
|------------|--|-----------------------|------|--|-----------------------|------|
| | | number | % | | number | % |
| Oct.—Dec. | 1319 | 1 | 0.08 | 5635 | 57 | 1.01 |
| Jan.—March | 382 | 1 | 0.26 | 4637 | 47 | 1.01 |
| April—June | 408 | 0 | 0 | 4037 | 30 | 0.74 |
| July—Sept. | 393 | 0 | 0 | 4583 | 6 | 0.13 |

The seasonal variation was calculated in the Odense material, comprising a total of 142 cases of pancreolithiasis on a 1 year basis. The frequency was highest in the period October through March, most clearly shown in the age group above 2 years of age, comprising the bulk of the material (Table 3). Fig. 1 shows graphically the distribution in the age group above 2 years of age. The curve demonstrates a maximum frequency during the late autumn and a minimum frequency during the summer.

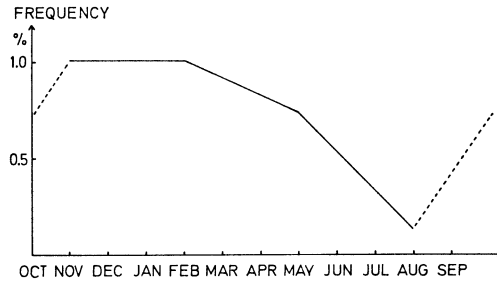


Figure 1. The seasonal distribution in the age group > 2 years (Odense).

Pathologic examinations

The isolated glands were without visible changes by inspection of the surfaces. By palpation the stone formations were easily found. After opening of the duct system the white stones were seen to distend the ducts completely, occasionally with varying intervals between rows of stones (Fig. 2). The smallest stones were always found in the narrowest ducts with a general

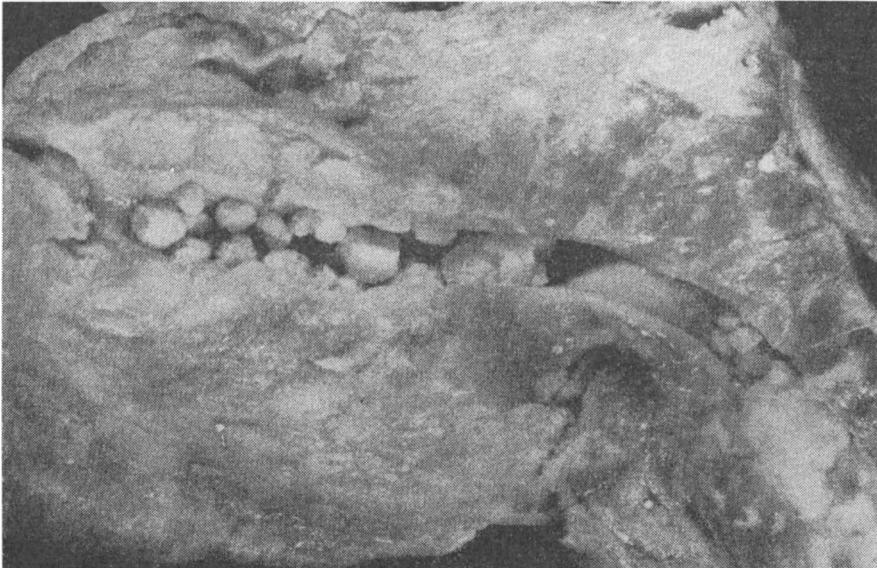


Figure 2. Part of a pancreas with a large opened duct containing stones.

increase in size through wider ducts in the direction of the main pancreatic duct as shown in the X-ray picture (Fig. 3). The ducts were dilated and the walls thickened. The lumen of the main secretory duct varied from normal up to 15—20 mm in diameter. In severe cases the walls could be up to 5 mm thick. Ulcerations in the mucosa extending down into the submucosa were sometimes present. In a few cases stones were found in the lumen of the duodenum. The glandular tissue did not show any changes. Microscopic examination showed a diffuse fibrosis of the lamina propria and submucosa of the ducts, which in connection with the dilatation had led to a more or less pronounced atrophy of the surrounding acini. The epithelial layer was most often without changes, but sometimes it had become atrophic or showed papillomatous proliferations. In the lamina propria histiocytic cell infiltration was often apparent. The v. Kossa-stained slides did not reveal any deposits of calcium in the interstitial tissue, in the secretory cells, in the acini or in the epithelial cells of the ducts.

The morphology of the stones varied considerably from case to case as well as in the individual case. They could be round



Figure 3. X-ray picture of a pancreas. The stones are found in the duct system.

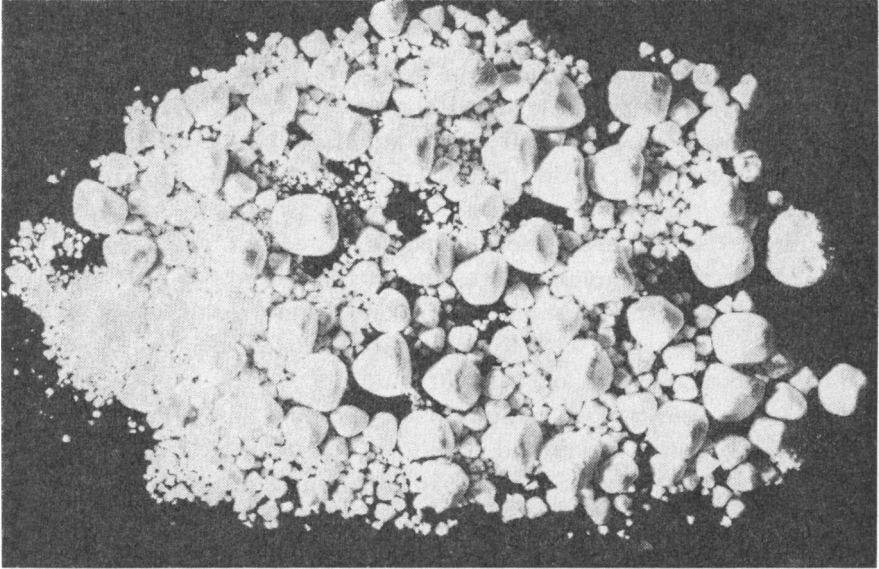


Figure 4. Collection of stones from a case of pancreolithiasis. Note the different sizes and forms.

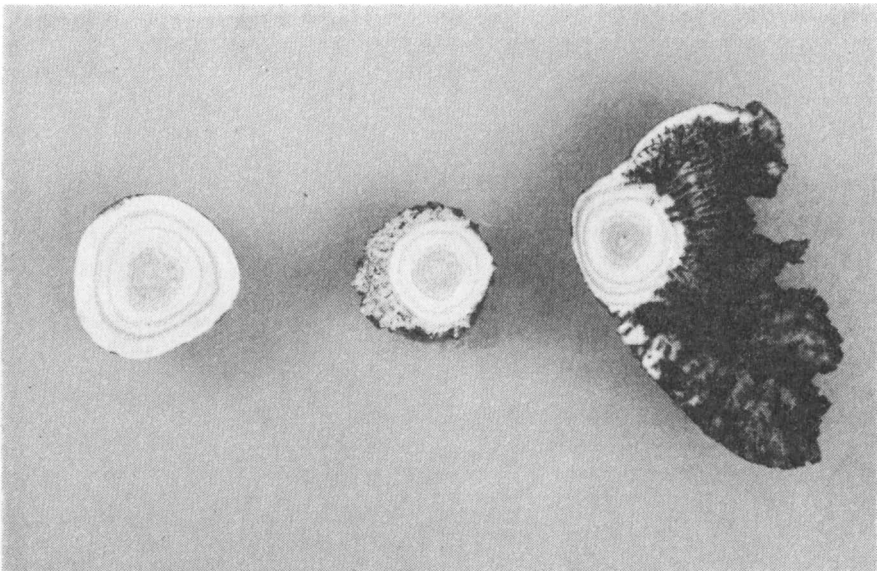


Figure 5. Polished and stained (Iron hematoxylin) cut surfaces. Note the concentric ring structures and the smooth (1, left) and rough surfaces (2, right).

or faceted and with a smooth or rough surface (Fig. 4). The diameter varied from 0.5 up to 15 mm. Individual stones could on one half of the surface be smooth, whereas the other half had irregular sharp prominences (Fig. 5). Polished cut surfaces showed concentric ring structures (shell stones) (Fig. 5).

The weight of the dried stones showed a case variation from below 1 g to 190 g. The average weight of stones in the different age groups is shown in Fig. 6. Only the 131 cases where pre-

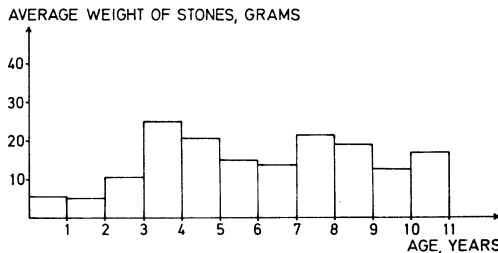


Figure 6. Diagram showing the average weight of stones in different age groups.

cise owner information was obtained are included. In Fig. 7 the average weights of stones are calculated in percentage of the average weight of pancreas in the different age groups. Both diagrams show the highest absolute and relative load of stones in the 3—5 years age groups.

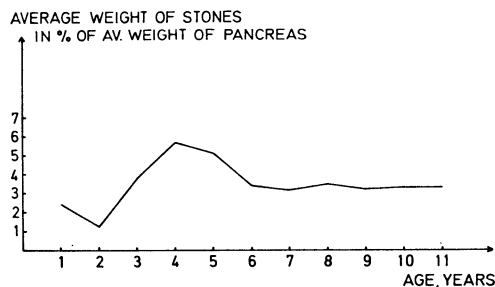


Figure 7. Average weight of stones in percent of average weight of pancreas in different age groups.

Chemical analysis

X-ray diffraction studies showed identical diagrams which exclusively contained lines, which all could be ascribed to calcium carbonate in the modification of calcite. It could

not be excluded that other substances were present, but these would have to occur in small quantities only or in poorly crystallized forms. The results of quantitative analysis for calcium, phosphate and magnesium are shown in Table 4. Average content of calcium was 38.1 % (36.4—40.9), phosphate 0.96 % (0.46—2.05) and magnesium 0.12 % (0.08—0.26). Of the 20 examined samples 14 were taken from females, 6 from males. Average content of phosphate was 0.85 % (0.46—2.05) in females, 1.31 % (1.17—1.47) in males. Average content of magnesium was 0.13 % (0.08—0.26) in females, 0.10 % (0.08—0.14) in males.

The calcium determination by Scheibler showed a content of 37.2 % and 37.9 %, respectively. Both results are within the

Table 4. Quantitative analysis of pancreatic stones.

| Accession no. | Sex | Age | Ca % | PO ₄ % | Mg % |
|---------------|-----|------------|------|-------------------|------|
| B 500 | ♀ | 1½—4 years | 40.9 | 2.05 | 0.13 |
| 771 | „ | > 4 years | 37.6 | 0.85 | 0.10 |
| B 747 | „ | „ | 36.4 | 0.80 | 0.26 |
| B 650 | „ | „ | 37.9 | 1.03 | 0.10 |
| B 835 | „ | „ | 36.7 | 1.01 | 0.15 |
| B 663 | „ | „ | 39.4 | 0.50 | 0.09 |
| B 832 | „ | „ | 39.1 | 0.76 | 0.16 |
| B 710 | „ | „ | 37.3 | 0.80 | 0.14 |
| 27 | „ | „ | 38.7 | 0.64 | 0.10 |
| 762 | „ | „ | 38.5 | 0.46 | 0.08 |
| B 622 | „ | „ | 37.6 | 0.84 | 0.12 |
| 763 | „ | „ | 38.1 | 0.97 | 0.13 |
| B 724 | „ | „ | 38.6 | 0.62 | 0.14 |
| B 528 | „ | „ | 37.4 | 0.78 | 0.16 |
| 32 | „ | „ | 39.1 | 0.59 | 0.10 |
| B 736 | ♂ | < 1½ years | 38.9 | 1.17 | 0.11 |
| 897 | „ | „ | 36.1 | 1.32 | 0.14 |
| 860 | „ | 1½—4 years | 38.2 | 1.34 | 0.10 |
| 900 | „ | < 1½ years | 39.1 | 1.47 | 0.08 |
| B 731 | „ | „ | 38.8 | 1.27 | 0.09 |
| Average | | | 38.1 | 0.96 | 0.12 |
| „ | ♀ | | | 0.85 | 0.13 |
| „ | ♂ | | | 1.31 | 0.10 |

limits of the above cited figures. The carbon dioxide determination by EDTA titration showed a content of 41.1 % and 41.8 % in the 2 samples examined.

DISCUSSION

The frequency of bovine pancreolithiasis has been calculated on a world basis by *Charvet* (1969) who found 1 case per 14,223 examined animals according to available literature and results of the French survey. A comparison of the frequency in France, England and Denmark during a parallel 3 months examination period (Oct.—Dec. 1969) showed the following figures: France, 1 case per 3,187, England 1 case per 3,982 and Denmark 1 case per 235 examined animals (*Velling & Vérine* 1971). It is noteworthy that systematic examinations disclosed a considerably higher frequency, about 4½ times in France, 3½ times in England and 60 times in Denmark compared to the hitherto known average frequency. Contrary to general belief pancreolithiasis seems to be a rather common condition in the bovine, but also with a remarkably high frequency in Denmark.

The frequency seems to rise with increasing age of the animals. *Charvet* found only 1 case per 9,674 among all slaughtered animals, but 1 case per 1,360 among adult cattle in the French material. In this material there was 1 case per 517 animals below 4 years of age and 1 case per 121 animals above 4 years of age. Both results indicate an approx. 5 times higher frequency among adult cattle compared with younger animals, although it can not be excluded that a sex factor also may be involved, due to the much higher frequency of females among the adult cattle (approx. 87 % in the Danish material). Among the cases with owner information the sex distribution was 3.5:1 in disfavour of the females. Thus the females might have a higher frequency than males, but further evidence to substantiate this possibility is not available (*Charvet*).

The breed distribution in the material is interesting because for the first time it shows a tendency toward a breed-related difference in the frequency. The Red Danish breed shows a higher frequency, whereas the Holstein-Friesian breed and particularly the Jersey breed show a lower frequency than calculated on basis of the average distribution of breeds in the country. Taking into account the predominance of the Red Danish breed in the country, this may partly, but not completely explain the

comparatively very high frequency of bovine pancreolithiasis in Denmark.

According to owners' information clinical symptoms were never detected during the course of the disease, at least not to an extent which had caused the owners to ask for veterinary assistance. This is in close agreement with the cases reported in the literature, where only 3 animals had shown symptoms related to an impairment of pancreatic functions with intermittent diarrhea, polydipsia and progressive cachexia (*Charvet*).

The uniform geographical distribution of the cases throughout the country indicates that possible predisposing factors related to the different soil conditions, e. g. difference in the mineral contents of the soil, particularly calcium and phosphate, are insignificant. The apparently lower frequency of cases in southern Jutland seems to be related to the lack of participating slaughter plants in this part of the country.

The seasonal variation in the frequency of pancreatic stones as demonstrated in the Odense material with maximum frequency in the late autumn and minimum frequency during the summer indicates that formation of pancreatic stones is a discontinual, fluctuating process, presumably followed by a partial ejection of stones into the duodenum. The rate of formation of stones might be influenced by the different crops used for consumption during the year, e. g. predominantly grass in the summer time and predominantly beets in the winter time.

The distribution and size of the stones in the pancreatic ducts combined with the shell stone structure can be explained as a result of a discontinual crystallization process, taking place during a slow passive movement through the duct system from minor to major ducts where they accumulate.

The dilatation, the ulceration or proliferation of the mucosa, and the fibrosis of the lamina propria and submucosa of the ducts are thought to represent secondary changes due to mechanical trauma, caused by the often rough surfaces of the stones. Apart from a more or less pronounced atrophy of the excretory acini close to the major ducts no other changes were found in the excretory or incretory parts of the gland.

The absolutely and relatively heaviest loads of stones in the gland were found in 3—5 years old animals with a slow decrease and then stabilization at a little lower level in animals up to 10—11 years. After a stronger action of the unknown patho-

physiological mechanisms leading to stone formation in the 3—5 years age groups the turn-over of formation and ejection may stabilize at a certain level, or it may reach a point with no further formation and ejection of stones.

The main constituent of the dried stones is calcium carbonate in the modification of calcite as was also found by X-ray diffraction studies of 7 samples in France (*Perrin et al.* 1969). A parallel examination and comparison of the chemical composition of 3 samples of stones from England, 3 from Denmark and 7 from France were made by *Vérine* (1971). The mean values of Ca, PO₄ and Mg did not show significant differences from the mean values on the 20 further examined samples in this material. Of these 20 samples, 15 were from females and 5 from males. No significant differences of the chemical composition of the stones were found in relation to the sex.

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SAMMENDRAG

Pancreassten hos kvæg i Danmark.

Resultaterne af en undersøgelse over forekomsten af bovin pancreolithiasis i Danmark fremlægges og diskuteres. Blandt 65.471 stk. kvæg slagtet på 9 eksportslagterier i 4. kvartal af 1969 fandtes 279 tilfælde. Frekvensen i det samlede materiale var 0,43 %, mens frekvensen hos dyr under og over 4 år var henholdsvis 0,19 % og 0,82 %. Der påvist en raceafhængig forskel i frekvensen, idet den var størst hos RDM, mindre hos SDM og mindst hos Jerseykvæg. Lidelsen syntes ikke at give anledning til kliniske symptomer, og der fandtes ingen korrelation med andre sygelige tilstande, som blev fundet ved kødkontrollen. Geografisk var tilfældene jævnt fordelt i landet.

På 1 eksportslagteri blev undersøgelsen udstrakt til også at omfatte de 3 første kvartaler af 1970. Der blev i dette materiale omfattende 142 tilfælde fundet en højere frekvens i det sene efterår og en lavere frekvens om sommeren.

Patologisk-anatomiske forandringer i de angrebne kirtler blev undersøgt makro- og mikroskopisk. De hvide, hårde, facetterede sten fandtes liggende i udførselsgangsystemet, og de akkumuleredes i hovedudførselsgangen, hvorfra der sker en vis udtømning i duodenum. Sekundære betændelsestilstande fandtes i udførselsgangenes epithel og ydre væg, mens der ikke fandtes signifikante forandringer i de inkretoriske eller ekskretoriske afsnit af kirtlen.

Kemiske analyser viste, at hovedbestanddelen var calciumkarbonat i modifikationen calcit, samt små mængder af fosfor og magnesium. En sammenlignende kemisk analyse af pancreassten fra kvæg i Frankrig, England og Danmark viste ingen signifikante forskelle.

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