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Spontaneous lesions in clinically healthy, microbiologically defined Göttingen minipigs

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Introduction

Different strains of miniature swine are gaining increasing interest as an alternative to other non-rodent species in pharmacological and toxicological research. Among these is the Göttingen minipig, which has been bred for these purposes for a number of years (*Glodek & Oldigs*, 1981). With the increasing use of these animals in experimental studies the necessity of available background material becomes urgent. The normal haematology and clinical chemistry have previously been investigated for Göttingen minipigs (*Ellegaard et al.*, 1995). When choosing and using the test animal it is important also to be aware of the spontaneous pathological changes that may be expected at examination.

Whereas studies have been published on the spontaneous pathology of the non-rodent species of choice at present, the Beagle dog *(Hottendorf & Hirth*, 1974), only few reports of spontaneous lesions in miniature swine have been published. These reports have been based on control animals from various studies, using miniature swine of the Troll strain (*Rinke,* 1997), and the Göttingen minipig (*Svendsen et al.,* 1998a). The latter study included only selected organs.

The aim of the present study was to provide a background material regarding spontaneous lesions in 3, 6, and 12 months old clinically healthy, microbiologically defined Göttingen minipigs reared under strict barrier conditions.

Materials and Methods

The study included 18 Göttingen minipigs obtained from the breeder, Ellegaard Göttingen Minipigs Aps, DK-4261 Dalmose. The animals were 3 of each sex from three age groups: 3 months (range 87-93 days), 6 months (178-190 days), and 12 months (358-371 days) old.

The animals had been bred and reared under full-barrier conditions and the microbiological status is monitored twice a year (*Hansen et al.,* 1997). Thus the animals in the present study should be free of 28 bacterial, 2 fungal, 8 viral and 5 parasitological agents commonly known as swine pathogens. The following agents have been detected in the herd: Porcine rotavirus and *Candida albicans*.

At 1 or 2 days of age all animals were given an injection of 250 mg/kg of iron-dextran (Imafer Vet. 100 mg FE/ml; Boehringer Ingelheim Agrovet, DK-2900 Hellerup) intramuscularly in the neck to prevent anaemia. The animals were group housed in floor pens with solid concrete at 2/3 of the surface and grids at 1/3 of the surface. They were fed restrictedly with an expanded pellet (SDS minipig diet, SDS, Witham CM8 3AD, UK) and water was available ad libitum. The temperature was 20-22° C , the relative humidity 50-70%, and ventilation intake (7-20 changes per hour) was through microfilters. The light/dark cycle was 12 hours.

The animals had a history free of clinical signs of disease and their haematology and clinical chemi-

stry were examined to further verify their health status. Immediately prior to shipment from the breeder blood samples were drawn from the bijugular trunc with the animals in dorsal recumbency, samples were cooled, and standard haematological and biochemical values as previously listed (*Ellegaard et al.*, 1995) were determined within 48 hours.

The haematological components were determined using the CellDynn 3500 automated analyser (Abbott). Blood smears were stained with Hemacolor (Merck, Darmstadt) and the differential leukocyte count was performed using the battlement method and fibrinogen assessed by the heat precipitation method (*Jain*, 1986).

Clinical chemical components were determined using a Cobas Fara automated analyser (Roche) and reagents from Roche (Basle, Switzerland) apart from calcium which was determined by atomic absorption spectrophotometry (Perkin Elmer) and sodium, potassium and chloride which were determined using the ion-selective electrodes on the Cobas Fara (Roche). Serum protein electrophoresis was performed using agarose gels (Paragon, Beckman) and ImageMaster densitometer and software (Pharmacia, Sweden).

Upon arrival at the university, the animals were anaesthetised by intraperitoneal injection of 2 ml/kg of a pentobarbital sodium solution (50 mg/ml) and killed by exsanguination via the axillary artery. A complete necropsy was performed and all abnormal macroscopic findings were noted. Samples for microscopy were taken from gross lesions as well as from the following tissues: Pituitary, thyroid, parathyroid, and adrenal glands, parotid and mandibular glands, tongue, pharyngeal tonsil, oesophagus, stomach (oesophageal, fundic, and pyloric part), duodenum, jejunum, ileum, caecum, colon, rectum, pancreas, liver, gall bladder, trachea, lung (cranial, caudal, and accessory lobe), ovaries, uterus, vagina, caudal mammary gland, testicles, epididymis, seminal vesicle, prostate, bulbourethral gland, kidneys, urinary bladder, heart (septum, right and left ventricle), thoracic aorta, mandibular and mesenteric lymph nodes, thymus, spleen, eyes, cerebral hemisphere, cerebellum, pons, medulla oblongata, thoracic and lumbar vertebral column including spinal cord, sciatic nerve, biceps femoris muscle, sternum, distal femur, and skin (thigh). Thus, the requirements of international guidelines were covered (*Anon.*, 1989).

Generally, tissues were fixed by immersion in a neutral buffered 4 % formaldehyde solution for a minimum of 24 hours. Eyes were fixed for 40 hours in Davidson's solution (containing 32% ethanol, 11.2% glacial acetic acid, and 6.6 % formaldehyde in distilled water) and subsequently stored in 70 % ethanol. Testes were fixed in Bouin's solution for 24 hours. Decalcification of osseous tissues after formaldehyde fixation was obtained by immersion in a solution containing 3.3 % formaldehyde and 17 % formic acid until a satisfactory texture was reached.

After fixation, trimming, and dehydration tissues were embedded in paraffin wax, sectioned at about 4 µm, and routinely stained with haematoxylin and eosin (H & E). The Luna's Biebrich scarlet (Luna's), periodic acid-Schiff (PAS), Perls' ferrocyanide (Perls'), and van Giesson's picrofuchsin stains were applied to selected parallel sections to demonstrate respectively, eosinophilic granulocytes, yeast, ferric iron, and collagenous tissue. Slides were evaluated blindly and any abnormality was recorded.

For the identification of yeast-like organisms seen in lesions in the periocular region of three animals, polyclonal rabbit-antibody against mannan from *C. albicans* (B459, DAKO, DK-2600 Glostrup), which is specific for *Candida spp.*, was used as the primary antibody in the three-layer horseradish peroxidase antiperoxidase (PAP) technique using a previously published protocol (*Jensen et al.*, 1993).

Results

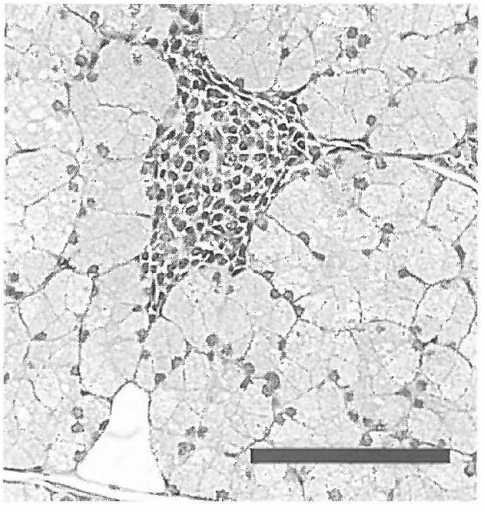
Compared to reference intervals (ref.int) for Göttingen minipigs (*Ellegaard et al.*, 1995), haematological or biochemical findings possibly associated with disease were recorded in one 3 months old male minipig with elevated ALAT (3.60 µkat/L, ref.int. 0.85-1.55), ASAT (1.84 µkat/L, ref.int. 0.22-0.78), CK (114.3 µkat/L, ref.int. 3.43-10.41), and LDH (49.2 µkat/L, ref.int. 12.98-19.99). One 6 months old female minipig had elevated ALP (10.10 µkat/L, ref.int. 1.85-5.87), and the three 3 months old female minipigs had

elevated ASAT (3.74 - 4.65 μkat/L, ref.int. 0.22-0.53).

At necropsy the following abnormalities were seen: In seven animals hyperkeratotic, yellowish discoloured areas were seen in the oesophageal region of the stomach. In one animal a bony swelling was observed at the osseous part of a rib. Three animals had brownish discoloured skin with crusts bilaterally in the periocular region. In one animal a single ovarian cyst was seen.

The by far most frequent microscopical lesion was focal mononuclear cell infiltrates, which were observed with varying frequency in all animals (1 to 6 incidences/animal). These infiltrates were dominated by lymphocytes with occasional macrophages and plasma cells. They were seen primarily in the interstitium (Fig. 1), peri- or paravascularily, and only rarely in the parenchyma of the organs. Such infiltrates were observed in the following tissues: Adrenal glands, cerebrum, epididymis, oesophagus, kidney, liver, lung, mandibular gland, meninges (cerebral, cerebellar and medullar), parotid gland, rectum, stomach (all parts), testes, tongue, and vagina.

Figure 1. Female, 12 months, parotid gland. Focal interstitial accumulation of mononuclear cells (H & E, 25 x obj., bar=100 mm).



A brief description of lesions other than mononuclear cell infiltrates is listed in Table 1 according to the organs, frequency, sex, and age distribution and a presentation of selected lesions is found in the figures 2-7.

Discussion

The biochemical abnormalities detected in a 3 months old male minipig (increased ALAT, ASAT, CK, and LDH) were consistent with a muscular disorder, and on microscopy this pig had focal myocyte necrosis in the lumbar region of the longissimus dorsi muscle. The nature and location of this lesion suggested focal trauma as the cause. The biochemical deviations detected in the three 3 months old female minipigs (increased ASAT) and in one 6 months old female minipig (increased ALP) could not be explained by any macro- or microscopic lesions.

The frequent finding of mononuclear cell infiltrates in the interstitium of various tissues is consistent with results from a study of the histopathology of selected organs in the Göttingen minipig (*Svendsen et al.*, 1998a). Such infiltrates are also commonly seen in other species and may indicate a normal immunological potential.

However, the occurrence of mononuclear cell infiltrates in the meninges of three animals and perivascularily in the cerebrum of two animals is unusual in clinically healthy animals. Their aetiology is obscure. The lesions were mild and strictly focal, and although similar changes occur in various infectious diseases, there was no evidence, clinically or pathologically, indicating systemic infection in the present cases. It should be noted, that similar lesions, in the absence of clinical disease, have been reported previously in this strain of pigs (*Svendsen et al.*, 1998a).

Most other inflammatory changes seen in the present study were in organs communicating directly with the environment, such as the eye, intestine, lung, mammary gland, and tongue. Except one (see below), none of these lesions could be related to the infectious agents known to be present in this strain of pigs or to other specific pathogens. On the basis of the location and nature of these lesions, they were assumed to be of a focal traumatic or non-specific infectious aetiology. However, in the periocular region of three 6 months old male pigs, focal exudative dermatitis was noted along with *Candida spp.*

Table 1. Table 1. Frequency of spontaneous lesions (mononuclear cell infiltrates not included) in Göttingen minipigs listed according to age and sex (Male/Female). Each age/sex group comprised 3 animals.

Organ	**Lesion**	**3 months**		**6 months**		**12 months**	
		M	F	M	F	M	F
Colon	Focal lymphoid follicle necrosis		1		1		
Eye	Focal retinal dysplasia		1				
	Focal lymphocytic keratitis		1	2			1
	Focal keratitis with neutrophils			1			
Kidney	Iron deposition *(fig. 2)*	3	3	2	2		
	Focal chronic interstitial nephritis					1	
	Subcapsular granuloma		1				
Liver	Iron deposition	3	3	1		1	1
Lung	Vacuolated alveolar macrophages *(fig. 3)*					1	1
Mammary gland	Focal, mild thelitis with neutrophils	1		1		1	
	Exudative galactophoritis						1
Mandibular lnn.	Focal eosinophilic inflammation			1			
	Iron deposition	3	3	2	3	1	1
Mesenteric lnn.	Iron deposition		1				
Muscle	Focal myocyte necrosis	1					
Ovary	Follicular cyst				1		
Prostate	Epithelial sloughing	1		1			
	Focal prostatitis with eosinophils *(fig. 4)*			1			
Rib	Old fracture	1					
Skin	Periocular hyperkeratosis and exudative dermatitis with *Candida spp.* in the superficial debris *(fig. 5)*			3			
Spleen	Focal follicular necrosis		1				
Stomach	Hyper-/parakeratosis *(fig. 6)*	2	2	3	3	2	3
	Microabscessation (lamina propria)	1	1	1			
	Focal necrotizing arteritis *(fig. 7)*			1			
	Focal perivasculitis with eosinophils					1	1
Thoracic duct	Microgranuloma			1			
Tongue	Focal dyskeratosis with infiltrating neutrophils		1				

Figure 2. Male, 3 months, kidney. Deposition of granules of ferric iron (arrowhead) in a glomerulus (Perls´, 40 x obj., bar=50 mm).

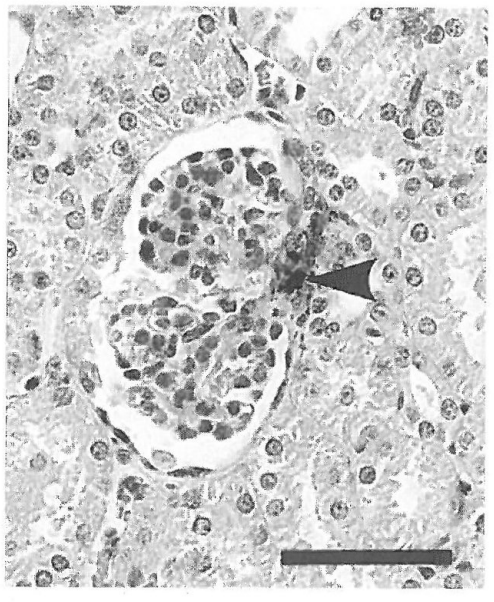


Figure 3. Male, 12 months, lung. Focal accumulation of vacuolated, alveolar macrophages (H & E, 40 x obj., bar=50 mm).

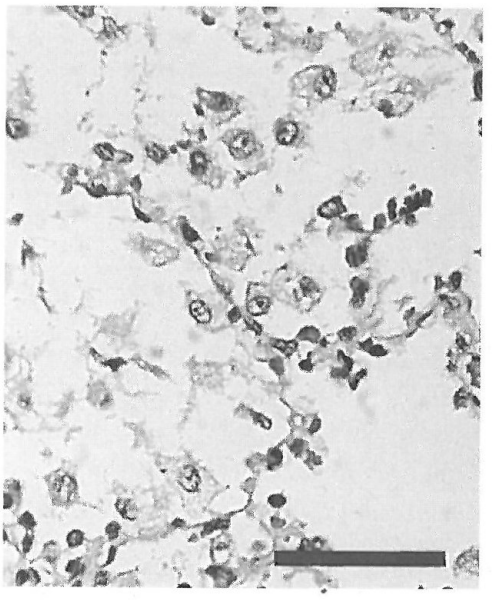


Figure 4. Male, 6 months, prostate. Focal prostatitis with mixed ductular exudate and periductular infiltrate containing eosinophils (arrowheads) (Luna´s, 25 x obj., bar=100 mm).

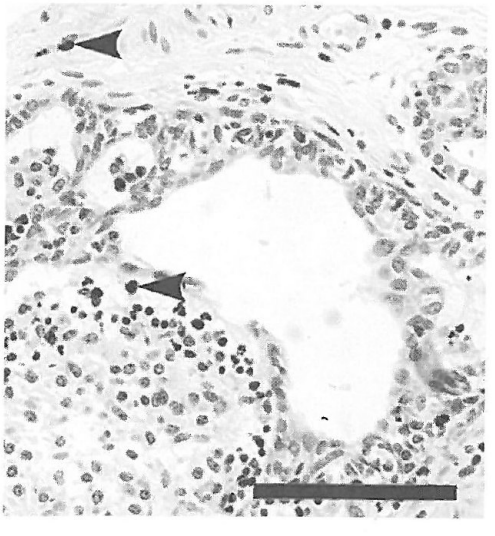


Figure 5. Male, 6 months, skin. Exudative dermatitis with hyperkeratosis and superficial *Candida spp.* (arrowhead) in the periocular region (PAP - immunohistochemistry, 40 x obj., bar=50 mm).

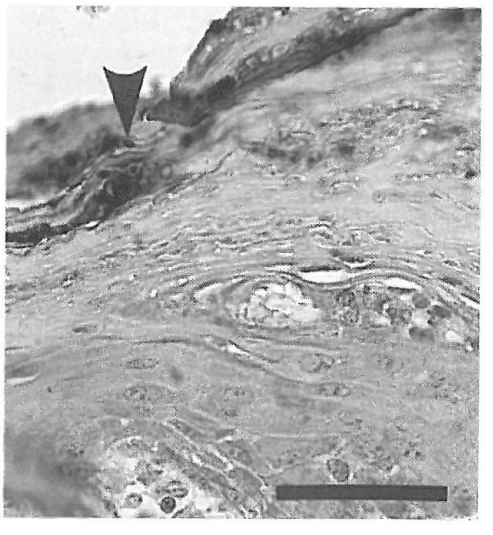
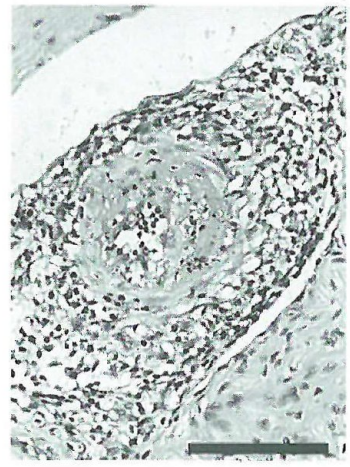


Figure 6. Female, 3 months, stomach (oesophageal part). Hyper- and parakeratosis, intraepithelial microabscesses (arrowhead) and inflammatory cell infiltrates in the lamina propria (H & E, 10 x obj., bar=150 mm).

Figure 7. Male, 6 months, stomach (oesophageal part). Focal necrotizing arteritis with a perivascular inflammatory infiltrate. (H & E, 25 x obj., bar=100 μm).



In other studies *Candida albicans* has been cultured from similar periocular lesions (*Bollen et al.*, 1998). Since *Candida spp.* are generally considered strictly opportunistic pathogens (*Odds*, 1988), it seems likely that infection was secondary to some unknown underlying factor.

Inflammatory lesions of the prostate are frequently encountered in laboratory rats, both as spontaneous (*Müntzing et al.*, 1979) and experimentally induced (*Gatenbeck*, 1986; *Northeved*, 1995) changes. Their pathogenesis is not entirely clear, but factors like age, stress, and drug-induced hormonal imbalance play a role. The cellular exudate in the reported cases in rats included neutrophils, macrophages, and other mononuclear cells whereas the lesion in the prostate of one of the pigs in the present study contained a large number of eosinophils. The morphology of the lesion in this animal does not allow for conclusions as to its aetiology and pathogenesis.

Focal necrotizing arteritis, as found in the stomach of one animal, has been reported to occur in the kidney, heart, lungs and spleen of this strain of minipigs (*Svendsen et al.*, 1998a). In Beagle dogs, similar lesions have been shown to occur spontaneously and an immune complex-mediated mechanism of injury has been suggested (*Kemi et al.*, 1990).

There was a high incidence of epithelial changes in the oesophageal part of the stomach of animals from all groups. Similar lesions are frequently observed in domestic swine and it has been shown that the incidence of hyperkeratosis is related to the feeding of finely ground rations (*Dobson & Davies*, 1978) and pelleted feed (*Pocock et al.*, 1969). The feed used in the present study was of a ground, heat-expanded, pelleted type which might explain the occurrence of the lesions.

The presence of iron deposits in the lymph nodes, liver, and kidneys is consistent with findings in previous studies in the Troll strain (*Rinke*, 1997) and the Göttingen minipig (*Svendsen et al.*, 1998b). In the latter study, the amount of iron deposition in the liver and kidney was shown to correlate with the dosage of iron-dextran administered to prevent anaemia. The present study supports the suggestion, that the incidence of iron deposition in the liver, kidney, and regional lymph node is declining with increasing age of the animals (*Svendsen et al.*, 1998b). However, the relatively low number of animals in each group in thepresent study does not allow for statistical

analysis. One animal in the present study had iron depo- sits in the mesenteric lymph node which is in agreement with the observation that iron deposition may affect lymph nodes in the whole body (*Rinke*, 1997).

Accumulations of vacuolated alveolar macrophages in the lung are important in toxicity studies, as certain drugs, such as basic amphophilic amines, induce such lesions (*Haschek & Witschi*, 1991). However, similar changes are known to occur spontaneously and the focal nature of the findings in the two animals in the present study suggests that they fall in this category.

Considering the limited number of animals examined, the present material should be regarded as a sample only. However, it does appear that spontaneous lesions are generally of a mild and focal nature in microbiologically defined, clinically healthy Göttingen minipigs reared under strict barrier conditions.

Summary

The use of miniature swine in biomedical research is increasing, whereas reports on their spontaneous pathology are sparse. Therefore, a study was performed to determine the incidence and nature of spontaneous lesions seen at necropsy and by microscopic examination. Eighteen clinically healthy, microbiologically defined Göttingen minipigs, reared under strict barrier conditions, were examined in a scheme covering both sexes and three different ages i.e., 3, 6, and 12 months. The most common microscopic lesion was focal accumulations of mononuclear inflammatory cells in various organs. Exudative dermatitis and hyperkeratosis with *Candida spp.* in the scaly debris was seen in the periocular region of three animals. Other inflammatory lesions were few, generally mild, and also of a focal nature. Iron deposition, probably due to preventive iron administration, was seen in the liver, kidneys, and lymph nodes. In the oesophageal part of the stomach a high incidence of apparently feed-related hyper- and parakeratosis was found. It is concluded that spontaneous lesions in microbiologically defined Göttingen minipigs are generally of a mild and focal nature.

Sammendrag

Brugen af minigrise i biomedicinsk forskning er stigende, mens undersøgelser af deres spontane patologi er fåtallige. Denne undersøgelse blev iværksat for at karakterisere hyppigheden og arten af spontane forandringer ved obduktion og mikroskopi. Atten klinisk raske, mikrobiologisk definerede Göttingen minigrise, opvokset under barriereforhold, blev undersøgt i et design omfattende begge køn og tre aldersgrupper, 3, 6 og 12 måneders grise. Den hyppigst forekommende mikroskopiske forandring var fokale ansamlinger af mononukleære celler i forskellige organer. Eksudativ dermatitis og hyperkeratose med *Candida spp.* i overfladen sås i huden omkring øjnene hos tre dyr. Herudover var der kun få inflammatoriske forandringer af mild og fokal karakter. Jernaflejringer, formentlig som følge af præventiv jernbehandling, sås i lever, nyrer og lymfeknuder. I ventriklens hvide del fandtes en hyppig forekomst af, formodentligt foder-relateret, hyper- og parakeratose. Det konkluderes, at spontane læsioner hos mikrobiologisk definerede Göttingen minigrise generelt er af mild og fokal karakter.

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