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intestines of PDV-positive seals suggests an increased turnover of FAE in these animals. We believe that PDV and/or other pathogenic organisms may have detrimental effects on FAE.

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# A CASE OF GLOMERULONEPHRITIS IN A DOG RESEMBLING IGA NEPHROPATHY IN MAN

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IgA nephropathy first described by Berger et al. in 1968 is being recognized increasingly as a common form of glomerulonephritis (GN) in humans (D'Amico, 1987). There is little documentation up to now about IgA nephropathy in dogs; high serum IgA values were found in dogs affected with several diseases such as pyodermia, demodicosis, aspergillosis, neoplasia, systemic lupus erythematosus, diabetes mellitus, rheumatoid arthritis, autoimmune haemolytic anaemia, idiopathic thrombocytopenia and Evan's syndrome.

In these subjects large numbers of circulating IgA bearing lymphocytes and a generalized IgA response within the tissues was found (Day and Phenale, 1988). Some of these changes were similar to those reported in Berger's disease of man. Salient clinical and pathological features of the disease in human patients are microhematuria, mesangial proliferative GN with IgA as the predominant Ig deposited, $C_3$ deposits, smaller amount of IgM and sometimes IgG deposits. The purpose of this study is to describe, in a German Shepherd dog, a case of nephropathy resembling Berger's disease in man.

## Material and methods

Kidneys from a 3-year-old, male, German Shepherd dog affected by *Leishmania infantum* were examined by light and electron microscopy. Immunohistochemistry and biochemical assays on urine and blood samples were also performed.

*Light microscopy:* For light microscopy renal tissue was fixed in 10% formalin buffered solution, embedded in paraffin and sectioned at 2 µm. The sections were stained with Hematoxylin-Eosin, Periodic Acid-Schiff and Periodic Acid Silver Methenamine (PASM). Five µm thick sections were stained with Congo-Red stain.

*Electron microscopy:* For electron microscopy specimens were fixed in 3% glutaraldehyde, postfixed in Osmium Tetroxide and embedded in Araldite. Thin sections were stained with uranyl and lead acetate and examined with an Elmiskope electron microscope.

*Immunohistochemistry:* Immunohistochemistry was performed on paraffin embedded sections using the immunoperoxidase technique of Sternberger. Antibodies used included rabbit anti-dog IgA, IgG, IgM and $C_3$. Specificity of labeling was demonstrated by omitting primary antiserum.

*Biochemical assays:* Serum chemistry analysis was performed to detect BUN, creatinine and total serum protein levels. Serum concentrations of IgA, IgG and immune complex were also determined. Urine samples were investigated for sediment analysis and quantitative and qualitative proteinuria was assessed by BioRad protein assay and SDS-PAGE electrophoresis respectively.

## Results

*Clinical findings:* Circulating immuno complex (77%I) and gamma globulin (6.5 g/dl) concentrations, particularly IgA fraction (0.6 g/dl), were significantly increased. Serum albumin level (2.55 g/dl) weas decreased while BUN (103 mg/dl) and serum creatinine (1.9 mg/dl) concentrations were increased. Marked glomerular non selective and tubular proteinuria (3.02 g/l), and microhematuria were present.

*Morphological findings:* Kidney lesion was predominantly a mild diffuse mesangial proliferative GN. Mesangium was widened by increased number of mesangial cells and mesangial matrix. A large number of infiltrating cells were found in the interstitium. Both lymphocytes and plasmacells were distributed in a diffuse infiltrate pattern with focal areas of dense cell collections mainly in periglomerular areas. Scattered foci of microscopic tubular hematuria were present. By means of immunohistochemistry IgA deposits were detected together with $C_3$, and occasional IgM and IgG deposits, and their distribution was mostly mesangial with the greatest intensity for IgA and $C_3$ and lesser for the other tested. Light microscopic changes were confirmed by electron microscopy that showed the presence of electron-dense mesangial and paramesangial deposits.

## Discussion

Our results are compatible with the previously reported on IgA nephropathy in humans particularly for the morphological and immunochemical aspect. IgA nephropathy is thought to be an immuno complex disease resulting from a poorly controlled mucosal immune response to environmental antigens to which the host is chronically subjected. Several exogenous antigens such as viruses or diet antigens have been implicated in IgA disease (Emancipator and Lamm, 1989). The case here reported was a dog affected by *Leishmania infantum* infection and a possible relationship between the presence of the parasite and an altered immunological response in the parasi-

tized host could exist. Different types of glomerulonephritis characterized by IgG, IgM and $C_3$ deposition have been already described in dogs and often associated to different pathological conditions.
This is the first description of an IgA nephropathy in a dog affected by a chronic parasitic disease. Although the morphological, electromicroscopical and immunological patterns are strongly resembling IgA nephropathy other attempts need to discriminate this GN from other immunological disturbances. IgA deposits in fact have been also found in proliferative lupus GN and other proliferative GN (Jennette, 1988) but the immunohistological demonstration of predominantly mesangial IgA immunostaining can be accepted as criteria for making a pathological diagnosis of IgA nephropathy.

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## CLENBUTEROL LONG-TERM ADMINISTRATION IN FINISHING FEMALE PIGS. NOTE 1: EFFECTS ON OESTROGEN AND PROGESTERONE RECEPTOR (ER AND PgR) DISTRIBUTION AND CONCENTRATION IN GENITAL TRACT

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Clenbuterol is a bronchodilator and tocolytic agent widely used in veterinary medicine. When this ß2-agonist is chronically administered, either orally or parentally, fat deposition is reduced (up to 30%) and protein accretion occurs (1). Therefore clenbuterol is illegally employed as «repartitioning agent» in animal husbandry, since it powerfully stimulates muscle growth in ruminants, pigs and poultry (2). Then, we noted that heifers longterm administered with clenbuterol at high dosages (our data not yet published), presented changes in the genital tract, consisting in an abnormal collection of mucus in uterus and vagina associated with ovarian alterations. Furthermore, ER and PgR progesterone receptor concentrations, investigated in different areas of the uterus, revealed a noteworthy increase. We have obtained similar results in female rats (3). Aim of our experiment was to verify if clenbuterol is able to induce analogous alterations in the reproductive system of female pigs.

### Material and methods

Eight Landrace x Large white female pigs, weighing about 120 kg, were randomly assigned to each of two experimental pelleted diets, containing 0 and 1 ppm of clenbuterol respectively. Animals were housed in two pens with slatted floor, in which they were exposed to existing photoperiod and ambient temperature. Feed and water were provided ad libitum. On the 48th day of treatment pigs were killed by exsanguination after a light electric shock. Blood samples were collected after 0, 24, and 48 days of treatment, in order to evaluate estrogen and progesterone serum levels, using RIA kits (Double Antibody Estradiol° and Coat-A-Count° DPC, USA). ER and PgR levels were measured by a modified DCC method on tissue samples (1 g of mucusa) collected from five different areas of the genital tract: vagina (A), cervix (B), body (C), first (D) and second (E) half of uterine horns. Steroid receptor concentrations, reported in Tab. 1 and Tab. 2, are expressed as femoles of bound hormone/mg of cytosol protein. Data obtained were statistically processed using the analysis of variance (two way with replication) and Duncan's test.

### Results and discussion

As reported in Tab. 2, the results revealed that clenbuterol affected both ER and PgR concentrations causing significant increases in treated animals ($p < 0.05$). The distribution of steroid receptors ranges from the lower values found in the vagina and in the cervix to the higher ones found in the uterus, mainly in the horns of either two groups. Besides, clenbuterol treatment never affected ER and PgR distribution along the genital tract (Tab. 1 and Tab. 2). Estradiol and progesterone serum levels, in accordance with macroscopical and histological observations, suggested a lack in ovarian activity in treated pigs. By contrast, in the control group, two subjects were in luteal phase, one in follicular phase and the remaining one in metestrus (Tab. 3).

Tab. 1: Effects of clenbuterol treatment on ER and PgR distribution and concentration in porcine genital tract (mean values ± ES)

| | TREATED | | CONTROLS | |
|---|---|---|---|---|
| | ER | PgR | ER | PgR |
| A) Vagina | 54± 23 | 96± 42 | — | 94 ± 22 |
| B) Cervix | 79± 33 | 369± 162 | 12± 0.6 | 117 ± 35 |
| C) Body | 268± 114 | 560± 168 | 25± 6 | 214± 885 |
| D) Horn (1st half) | 304± 89 | 828± 176 | 41± 6 | 290± 120 |
| E) Horn (2nd half) | 227± 81 | 558± 44 | 55± 3 | 388± 98 |

Tab. 2: Statistical evaluation of results

| | Treatment (T) | | Samples (S) | | | | | Interact | |
|---|---|---|---|---|---|---|---|---|---|
| | control | clen | A | B | C | D | E | F values (T) | (S) |
| ER | 27a | 186b | 27a | 46a | 146b | 172b | 141b | 20.92** | 2.82* 1.55 |
| PgR | 221a | 482b | 95a | 243a | 387b | 559b | 473b | 14.01** | 5.59** 1.63 |

*P<0.05; **P<0.01; a,b P<0.05.