PROTEINURIA IN DOGS WITH PYODERMA

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SUMMARY

Agarose gel electrophoresis was used for fractionation of urine proteins obtained from 39 dogs with pyoderma. Thirty dogs (77%) had earlier on tested positive for proteinuria by reagent strips. Eighteen of the 30 dogs (46.2%) showed electrophoretic patterns consistent with albuminuria and was similar to patterns demonstrated in dogs with histologically confirmed glomerulonephritis. Three dogs (7.7%), showed albuminuria and increases in globulins thus giving a pattern resembling physiologic proteinuria. This was considered to be a variant form of glomerular proteinuria. Eight dogs (20.5%) had an electrophoretic pattern characterized by low albumin and high beta globulins as well as some increase in alpha 2 globulins thus resembling tubular proteinuria in humans. These findings indicate presence of glomerulonephrotic lesions in a high proportion of dogs with pyoderma. It was concluded that kidney function tests are worthwhile in dogs with pyoderma.

INTRODUCTION

Staphylococcal pyoderma is the most common skin disorder of the dog (Muller et al., 1989; Manson, 1991). Being among the importnat chronic skin diseases, pyoderma is theorized to be one of the many causes for glomerulonephritis (Grauer, 1992). The possible mechanism in the development of pyoderma induced glomerulonephritis is the one involving immune complexes which culminates in glomerular injury (Grauer, 1992). Proteinuria which occurs following injury to the glomerular barrier is one of the most valuable indicators of kidney disease (Beetham and Cattell, 1993). Glomerular proteinuria which is the commonest type of proteinuria is caused by increased glomerular permeability to proteins, particularly albumins, either due to primary flomerular disease or secondarily following systemic illness (Schaeffer and Del Greco 1992). Therefore, albuminuria as well as the loss of other high molecular weight proteins are characteristic features of glomerulonephritis (Chiggeri, 1989).

Tubular proteinuria is another form which tends to involve proteins that in normal situations would be filtered but later reabsorbed in the region of proximal convoluted tubules. The appearance in urine of such proteins occurs following impaired tubular reabsorption as caused by disorders in the interstitium and/or tubules (Schaeffer and Del Greco 1992). Alteration in renal hemodynamics coupled with increased filtration of normal proteins causes yet another type of proteinuria. This type is called physiological proteinuria (Schaeffer and Del Greco 1992). Physiological proteinuria is transient and occurs in absence of renal disease.

Chronic bacterial infections of the skin have been implicated in the causation of renal disease (Polzin et al., 1992). Pyoderma, a chronic bacterial disease of the

skin is frequently diagnosed in dogs (Muller et al., 1989). However, there is paucity of information on the effect of this disease on kidney function.

To establish a non-invasive and sensitive program for the clinical monitoring of renal involvement in dogs with pyoderma, urine protein electrophoresis was investigated.

MATERIALS AND METHODS.

Animals

Two hundred dogs presented at the Small Animal Hospital of the Royal Veterinary and Agricultural University, Copenhagen, were examined for signs of pyoderma and/or overt kidney disease. These were both male and female dogs between 1-13 years and of different breeds. Thirty nine dogs with pyoderma were used as cases. Ten dogs with histologically confirmed glomerulonephritis were used as proteinuria positive controls. Twenty five clinically healthy dogs were used as proteinuria negative controls.

Sample collection

From each dog 10 ml of urine was collected by antepubic cystocentesis. Immediately after collection urinalysis involving the measurement of specific gravity by refractometer, use of multistix (Bayer diagnostics) for various biochemical components and examination of the urine sediment following centrifugation of the urine sample at 1000 rotations per minute for 5 minutes was undertaken. the supernatant was poured into a Nanosep urine concentrator (Intersep Filtration Systems, Berkshire, UK) for its concentration, usually 200 times. The concentrated urine was dispensed into labelled 0.5 ml eppenddorf plastic tubes and stored at - 20°C until electrophoresis was collected, serum obtained and stored at - 20°C until electrophoresis was carried out.

Cases suspected to have kidney problems on account of high blood urea levels and signs of renal failure were euthanised and kidney samples for histopathology collected and sent to Pathology department for further work.

Electrophoresis

Five microlitres of the concentrated urine were applied to Beckman Paragon SPE Agarose gels (Beckman Instruments, Brea, California) and allowed to diffuse in for 5 minutes. Electrophoresis was carried out at 100 V for 25 minutes in barbital buffer, pH, 8.6 and 0.05 ionic strength. Staining and destaining was carried out according to Beckman Instruments instructions. After complete drying of the gels, densitometric quantification of the fractionated proteins was done using a 600 nm Beckman Appraise™ Densitometer (Beckman Instruments, Inc., Brea, California). The fractionated protein bands were identified by comparison to serum electrophoretic profiles obtained from the same animals.

RESULTS

Of the 200 dogs examined, 39 were found to have different forms of pyoderma (Table 1). Agarose electrophoresis of urine from these cases revealed that albumin was the main protein lost in 18 dogs (46.2%). Densitometric scanning of the electrophoretograms in this category of dogs showed the albumin loss to be in 58.7% to 72.2% range and the albumin to globulin (A/G) ratio ranging between 1.42 and 2.65 (Fig. 1). For this type of proteinuria the average (A/G) ratio was 1.71, with a standard deviation (SD) of 0.25.

Figure 2 shows a slightly variant

pattern, in which despite the predominance of albumin loss, there was also an increase in loss of the alpha and beta globulins. The A/G ratio for this pattern was 0.35 and was seen in long standing cases of pyoderma.

In another pattern demonstrated in 8 dogs (20.5%) the A/G ratio was as low as 0.13, with a SD of 0.004. In 2 of these 8 dogs the proportion of beta (46.4%) and alpha (43.7%) globulins lost in urine was substantially higher than that of albumin (3.5%) as displayed in figure 3. This pattern resembled human tubular proteinuria.

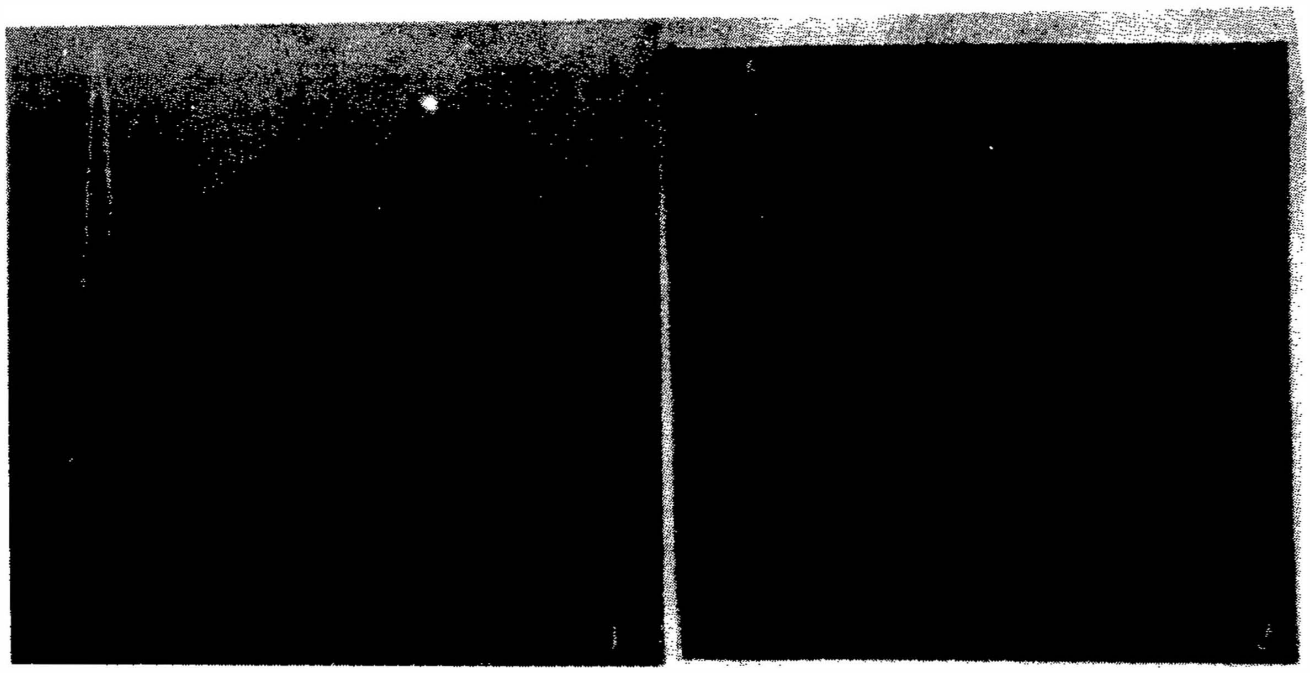
A pattern resembling human physiological proteinuria was found in 3 cases (7.7%). This form had an A/G ratio of 0.48 and 0.007 Sd (Fig. 4). The remaining 10 dogs (25.6%) did not fall neatly under any of the above patterns. Among the 25 negative controls, 3 dogs had proteinuria demonstrable by multistix.

As for the positive controls, densitometric quantification of the electrophoretograms revealed high A/G ratios (> 2.0) indicating significant albumin loss in those cases. Histopathologic features of these cases indicated the presence of glomerulonephritis.

DISCUSSION

Results of this work indicate that dogs with pyoderma are likely to suffer from renal involvement. Of the 39 proven pyoderma cases, 30 (77%) had significant proteinuria. That an overwhelming majority of those cases displayed prominent albumin peaks clearly indicates glomerular pathological changes as reported by Lulich and Osborne (1990). A recent study on dogs with Ehrlichiosis (Codner et al., 1992), reported that most cases with proteinuria do not have demonstrable glomerular lesions. These authors were of opinion that these were cases with minimal glomerulonephropathy. In the present study though, there was more than one electrophoretic pattern for the glomerular type of proteinuria, which indicates that there are is more than one type of kidney lesions probably depending on the type and seriousness of pyoderma. In the first pattern, there was significant albuminuria, A/G ratio > 1.52, these were considered to be cases of glomerulonephritis. Results for this group of dogs were in agreement with the findings in a positive control group in which histological confirmation was done (Fig. 5). Another pattern was the one with moderate to low albuminuria, A/G ratio around 1. This is the group which appears to conform to the findings of the study by Lulich and Osborne (1990). These are, most likely early cases of glomerulonephritis which may not show obvious lesions on histological section at this point in time as also reported by Grauer (1992).

Fig. 1: Urine electrophoretic pattern for dogs with pyoderma. Note high levels of albumin loss compared to globulin losses in urine, it is a typical glomerular proteinuria pattern. Alb: Albumin, α: Alpha, ß: Beta and t: Gamma globulins. **Fig. 2**: Urine electrophoretic pattern for long standing cases of deep pyoderma. α: Alpha, ß: Beta and t: Gamma globulins. **Fig. 3**: Urine electrophoretic pattern from pyoderma cases showing a high proportion of alpha and beta globulin losses in urine, also note subdued albumin level. The pattern represents tubular proteinuria. α: Alpha, ß: Beta and t: Gamma globulins. **Fig. 4**: Urine electrophoretic pattern from pyoderma dogs. Note resemblance with physiological proteinuria in humans. α: Alpha, ß: Beta and t: Gamma globulins.



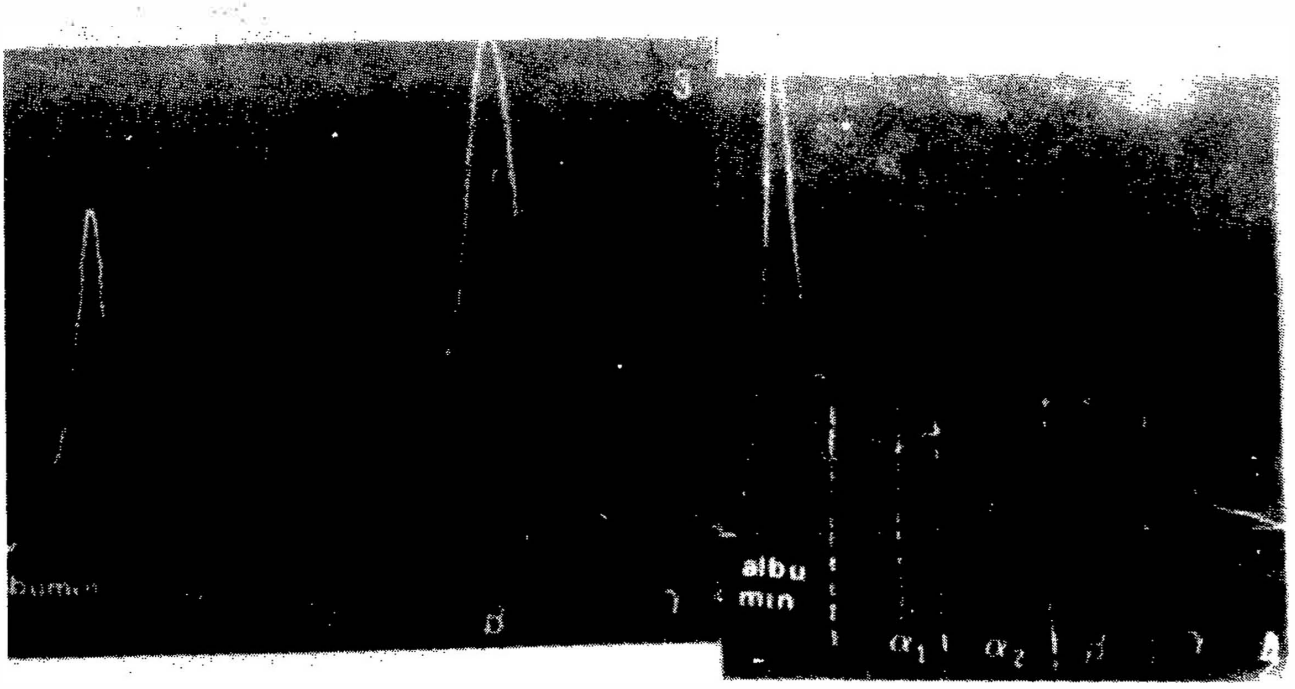

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Table 1. Dogs with different types of pyoderma

Dog no.	Age in years	Type of pyoderma	Dog no.	Age in years	Type of pyoderma
1.	7	G.S. Deep	21	7	G.S. deep
2.	8	G.S. Deep	22	9	Superficial
3.	8	Deep	23	5	G.S. deep
4.	8	Furunculosis	24	5	Superficial
5.	8	Superficial	25	8	G.S. deep
6.	3	Superficial	26	7	Superficial
7.	1	Superficial	27	10	Superficial
8.	1.5	Folliculitis	28	7	Superficial
9.	1.5	Superficial	29	1.5	G.S. deep
10.	8.5	Superficial	30	12	Frunculosis
11.	4	Superficial	31	8	Interdigital
12.	13	Folliculitis	32	13	Deep
13.	7	Superficial	33	7	Superficial
14.	7.5	Superficial	34	9	Deep
15.	10	Interdigital	35	4	G.S.deep
16.	6	Superficial	36	2	Superficial
17.	8	Superficial	37	10	Deep
18.	4	Folliculitis	38	1	Deep
19.	8	G.S. Deep	39	8	Deep
20.	9	Superficial			

G.S. Deep pyoderma: German shepherd deep pyoderma.

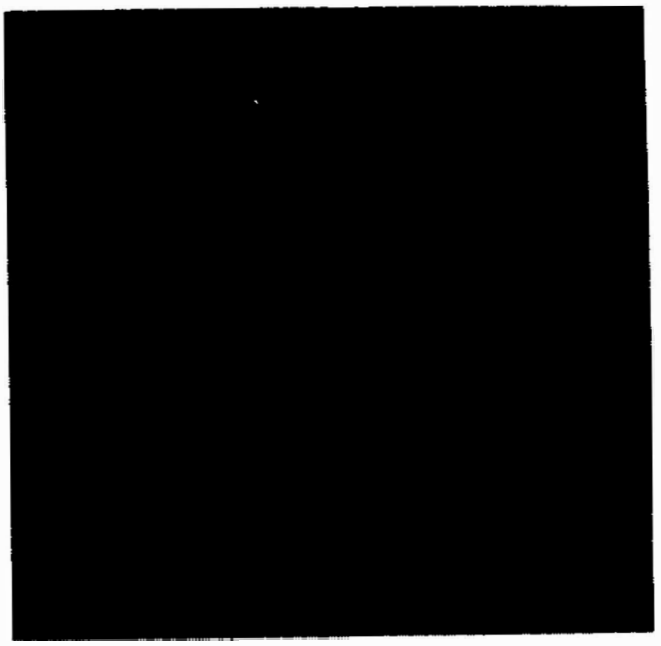

Figure 5: Comparison of urine electrophoretic pattern from pyoderma cases to that of dogs with chronic renal failure. Dotted line represents pyoderma cases and solid line is for chronic renal failure. Alb: Albumin, α: Alpha, ß: Beta and t: Gamma globulins.

Cases which were found with significant losses of albumin and globulins with high molecular weight, were thought to have severe glomerular lesions as reported by Lulich and Osborne (1990). Most were long standing cases of deep pyoderma. Their number was small. This sheds light on the consequences of pyoderma in kidney performance. The dogs which had a physiologic proteinuria like pattern could indicate the presence of a variant form of glomerular proteinuria. It is possible in this pattern, transferrin and IgG with other globulins are lost in urine in quantities. This may be due to increased glomerular permeability resulting from loss of negative charge from glomerular basement membrane and structural damage to the basement membrane (Beetham and Cattell, 1993).

In almost all cases, pyoderma is secondary to other contributing conditions e.g. Demodicosis and other parasites, Hypothyroidism, Hyperadrenocorticism and immunosuppressive chemotherapy (Muller et al., 1989). All these conditions have a potential for causing glomerulonephritis in small animals (Grauer, 1992). Long term antibiotic therapy has proven to form a cornerstone of therapy for pyoderma (Manson, 1993). Unfortunately in some cases this approach does not work and corticosteroid therapy remain the only viable option (Muller et al., 1989).

Corticosteroids exacerbate glomerular lesions and proteinuria (Granuer, 1992).

In conclusion, proteinuria principally albuminuria was found to be common in dogs with pyoderma. This supports the theory on causation of immune-complex glomerulonephritis in dogs with chronic skin diseases (Polzin et al., 1992). Finding of the same electrophoretic pattern in histologically confirmed cases of glomerulonephritis, served as proof for presence of relationship between pyoderma and immune complex glomerulonephritis. This indicates that it is important to include tests for kidney function in dogs with pyoderma.

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