Mass screening of Irish wolfhound puppies for portosystemic shunts by the dynamic bile acid test

M. G. KERR, T. VAN DOORN

Five hundred and sixty-six Irish wolfhound puppies aged six to 15 weeks were tested for congenital portosystemic shunts by the dynamic bile acid method. Plasma ammonia concentration was also measured in 165 of the puppies both fasting and postprandially. Nineteen puppies (3·4 per cent), nine males and 10 females, had portosystemic shunts. Smaller litters appeared to be more likely to contain affected puppies. The postprandial bile acid concentration was a reliable predictor of the presence of a shunt, with the highest concentration in a normal puppy being 38 µmol/litre and the lowest in an affected puppy being 43 µmol/litre. In contrast, fasting bile acid concentrations were normal in the majority of the affected puppies. There was considerable overlap in fasting plasma ammonia concentrations between normal and affected puppies (26 puppies, 15·8 per cent of those tested). Postprandial ammonia concentration appeared to give better separation between the two groups, apart from two individuals which produced bizarre results. It was concluded that the postprandial or dynamic bile acid test is an appropriate test for the mass screening of Irish wolfhound puppies for portosystemic shunts, and guidelines are proposed for the interpretation and follow-up of the test.

CONGENITAL portosystemic shunts occur only sporadically in dogs, but their effects can be extremely distressing to the animal's owner. The clinical signs are often vague or imperceptible in dogs less than 12 weeks, old and it is most common for the new owners of a young puppy to notice poor weight gain, lethargy and vague neurological signs. The condition is believed to be inherited in a number of breeds, most notably the Irish wolfhound (Meyer and others 1995).

The measurement of venous plasma ammonia concentration has been shown to have some predictive value for portosystemic shunts in the Irish wolfhound (Meyer and others 1996). However, the technique has disadvantages, particularly the necessity of taking the puppies to the laboratory to be blood sampled, because blood ammonia is so unstable that samples can be preserved in ice for only comparatively short periods. One analyser marketed to veterinary practices does have an ammonia kit available, but its results have been shown to correlate poorly with the reference method (Mischke and others 1992). In addition, Meyer and others (1996) have shown that the ammonia concentrations of normal and affected dogs overlap, and it may therefore be difficult to decide how to treat puppies with values close to the cut-off level. In contrast, samples for the measurement of bile acids may be posted to the laboratory without appreciable deterioration, and experience has suggested that normal and affected individuals might be distinguished more satisfactorily by this method (Kerr 1996).

The dynamic bile acid test is based on the enterohepatic recirculation of bile acids which, in the normal individual, does not involve the peripheral circulation. Bile acids are synthesised in the liver, then conjugated and excreted in the bile into the duodenum where they assist the digestion of fat. Subsequently they are reabsorbed in the distal small intestine and returned to the liver via the portal vein. Thus, in the one to two hours after a meal there is a marked increase in the bile acid concentration of portal venous blood, but in normal animals the bile acid concentration in peripheral venous blood increases only slightly if at all. However, where there is a direct communication between the portal and systemic circulations, the concentration of bile acids in peripheral blood increases markedly.

In 1996, a project was begun in association with the Irish Wolfhound Club and the Irish Wolfhound Society with the aim of screening as many puppies as possible for portosystemic shunts. All the puppies were tested by the dynamic bile acid method, and when it was practical to bring the litter to the laboratory, ammonia was also measured, both fasting and postprandially. This paper reports the results from the first two-and-a-half years of the screening programme.

MATERIALS AND METHODS

From early 1996 breeders of Irish wolfhound puppies were encouraged, through the breed societies, to have their puppies screened before sale. They were usually screened at six to eight weeks of age, although some litters were a little older (up to 15 weeks). In total, 566 puppies from 88 litters were tested, with litter sizes ranging from one to 13 puppies. Breeders who lived within reasonable travelling distance brought the puppies to the laboratory so that ammonia could be measured in addition to the bile acids (165 puppies from 27 litters). Breeders from further afield took the puppies to their veterinary surgeon for sampling, and only bile acid measurements were made. A blood sample was taken after an overnight fast, the puppies were then fed their usual morning meal, and a second blood sample was collected 90 to 120 minutes later.

Blood for ammonia measurement was collected into EDTA, immediately refrigerated, centrifuged within 20 minutes of collection, and the plasma separated and analysis carried out within three hours. Blood for bile acid measurement was collected into plain glass vials, allowed to clot, and the serum separated. Samples collected at the laboratory were analysed the same day, and samples sent by post were analysed on the day of receipt.

Ammonia was measured by the 2-oxoglutarate method (procedure 171-UV; Sigma Diagnostics), and bile acids were measured by nitro blue tetrazolium method (procedure 450; Sigma Diagnostics). Both measurements were made on a Cobas Mira random access analyser (Roche Instruments).

For the purpose of this study the classification of the puppies as affected or unaffected has been based on the results of a clinical follow-up, rather than solely on the results of the biochemical tests. When a puppy was suspected of having a shunt, the breeders were informed, and a decision was made as to whether euthanasia or surgery should be performed. Before euthanasia, a second test was always carried out to

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TABLE 1: Fasting and postprandial concentrations of ammonia and bile acids in normal Irish wolfhound puppies and in puppies with portosystemic shunts

	Range of values in normal puppies (median value)	Range of values in affected puppies (median value)	Normal puppies above lowest affected (%)	Affected puppies below highest normal (%)
Fasting ammonia (µmol/litre)	31 to 286 (102)	167 to 360 (257)	19 (12%)	7 (78%)
Postprandial ammonia (µmol/litre)	38 to 568 (114)	97 to 549 (410)	101 (65%)	9 (100%)
Change in ammonia (µmol/litre)	−169 to +456 (15)	−70 to +273 (143)	144 (92%)	9 (100%)
Fasting bile acids (µmol/litre)	0 to 29 (11)	9 to 50 (18)	331 (61%)	14 (74%)
Postprandial bile acids (µmol/litre)	1 to 38 (14)	43 to 390 (113)	0 (0%)	0 (0%)
Change in bile acids (µmol/litre)	−9 to +25 (3)	+26 to +368 (93)	0 (0%)	0 (0%)

ensure that the puppy had been classified correctly. In all but three cases the presence of a shunt was confirmed postmortem. The three exceptions were all showing clear clinical signs of hepatic encephalopathy and the diagnosis was not in doubt. In all 10 cases which were referred for surgery, the diagnosis was confirmed by the referral centre and the shunts were demonstrated at surgery (White and others 1998). In one case an affected puppy was initially missed because the owner inadvertently brought the wrong puppy for retesting after a suspect result. Otherwise, none of the puppies classified as unaffected was subsequently shown to have a shunt. One such puppy died of an unidentified liver disease, but an independent postmortem examination revealed no evidence of a shunt (T. J. Whitbread, personal communication).

RESULTS

Altogether 19 puppies (nine dogs and 10 bitches) were found to have portosystemic shunts during the two-and-a-half years of the study, which represents 3·4 per cent of the puppies tested. Three litters each contained two affected puppies, so that only 16 of the 88 litters tested (18 per cent) contained affected puppies. In general it appeared that smaller litters were more likely to contain affected puppies; the mean (sd) size of the affected litters was 4·9 (2·1) puppies (range one to eight), compared with 6·9 (2·5) puppies for the unaffected litters (range two to 13). Some of the affected puppies were considered to be clinically abnormal by the breeder but this was not always so, and in two cases the affected puppy was described as 'the pick of the litter' and had already been selected to be retained for breeding.

All the shunts explored either surgically or postmortem were of the left divisional type draining into the left hepatic vein, consistent with a persistent ductus venosus (Payne and others 1990, Lamb and White 1998, White and others 1998).

The fasting and postprandial levels of ammonia and bile acids, and the change in both analytes with feeding, are shown in Fig 1, and the median and range of results for each category are shown in Table 1, together with the numbers involved in any overlap between the normal and affected puppies in each category.

One anomalous result should be highlighted. A litter of six puppies (litter MV) was tested, but only postprandial samples were recieved by the laboratory. One puppy had a doubtful result, with a bile acid concentration of 38 µmol/litre. The practice then submitted samples for the dynamic test for the suspect puppy and another, which, although having a bile acid concentration of only 16 µmol/litre on the first test had exhibited some abnormal behaviour. On the second test both puppies were clearly abnormal, the originally 'normal' puppy showing an increase from a fasting level of 9 µmol/litre to 54 µmol/litre postprandially, and the suspect puppy showing an increase from 10 µmol/litre to 92 µmol/litre. On enquiry, it was discovered that during the initial testing only a small amount of food had been put down in a pen containing all six puppies, and they had not been observed to ensure that all of them had eaten. It was therefore thought possible that one puppy, perhaps feeling unwell, had not eaten and its 'postprandial' sample was in fact a fasted sample. As a result of the uncertainty the entire litter was retested, with careful attention to the amount of food consumed by each puppy. The results of that test confirmed that these two puppies were

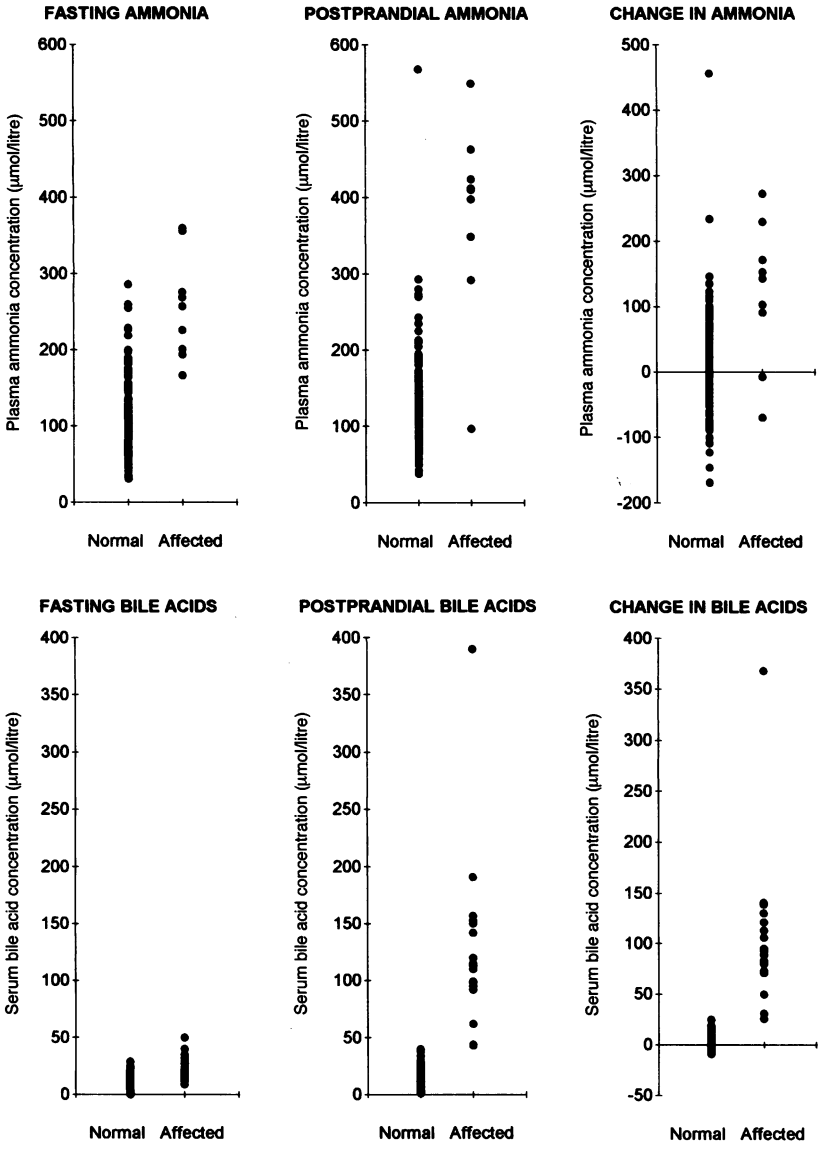

FIG 1: Fasting and 90 to 120 minutes postprandial ammonia and bile acid concentrations, and the change in both analytes with feeding, in normal and affected Irish wolfhound puppies. For the measurements of ammonia there were 156 normal puppies and nine affected puppies; for the measurements of bile acids there were 547 normal puppies and 19 affected puppies

TABLE 2: Standard interpretation of the dynamic bile acid test for distinguishing between Irish wolfhound puppies with or without portosystemic shunts

	Normal puppies	Affected puppies	Doubtful puppies
Postprandial bile acids (μmol/litre)	<30	>50	30 to 50
Change in bile acids (μmol/litre)	<20	>25	20 to 25
Results similar to littermates?	Yes	No	No

indeed affected and the other four were normal. It is the results from this re-test which are included in the statistical analysis of the study. In all other cases in which a puppy was retested, it is the first set of results which has been included.

DISCUSSION

Ammonia levels

Hyperammonaemia as a 'normal' occurrence in young wolfhound puppies has been reported by Meyer and others (1995, 1996). The normal puppies in this study had an even wider range of fasting ammonia concentrations, with 29 (18 per cent) having levels above 150 μmol/litre, the upper limit of normal quoted in the 1995 paper, and 49 (31 per cent) having levels above 125 μmol/litre, which was suggested as the criterion for distinguishing a dog with a shunt in the 1996 publication. In addition there was a larger overlap between the normal and the affected puppies in this study than was observed by Meyer and others (1995, 1996). The overlap figures for the postprandial ammonia concentration are markedly skewed by two puppies which had extreme postprandial results – one normal puppy with a postprandial ammonia concentration of 568 μmol/litre (fasted concentration 112 μmol/litre) and one affected puppy with a postprandial ammonia concentration of only 67 μmol/litre (fasted concentration 167 μmol/litre). If these two results are omitted the postprandial ammonia concentration did provide quite good discrimination between the normal and affected puppies, with only a small overlap (two puppies) in the 290 to 295 μmol/litre range. Nevertheless, the two anomalous results reduce confidence in this measurement. Calculating the change in the ammonia concentration after a meal appeared to be of no benefit in distinguishing normal from affected puppies, even if the two anomalous puppies were omitted.

This 'transient metabolic hyperammonaemia' of Irish wolfhound puppies has been shown to decrease substantially by 13 weeks of age and to be entirely absent in the adult dogs (Meyer and others 1996). Many of the puppies in this study were only six weeks of age, as opposed to the seven to eight-week-old puppies studied by Meyer and others. This may to some extent explain the finding of even higher concentrations in some normal puppies. There is no doubt that this peculiarity of the breed makes it very difficult to interpret the ammonia results in young puppies, particularly when breeders wish the test to be carried out as early as possible. However, ammonia is in any case notoriously difficult to measure, in part because of its labile nature, and even among the older puppies the present results differ from those of Meyer and others (1996). It therefore appears that the single fasting ammonia measurement is not always a reliable screening tool for the identification of affected puppies.

Bile acid levels

As with the fasting ammonia measurements, the fasting bile acid concentrations of the normal and affected puppies overlapped substantially. However, the postprandial bile acid levels differentiated clearly between the normal and affected puppies. In addition, the change in the bile acid concentration with feeding also resulted in a clear, though narrow margin of separation between the two groups. It was concluded that the bile acid measurements provided a reliable indicator of the presence or absence of a portocaval shunt.

At the end of the first year of the study the interpretation of the test was standardised as shown in Table 2. Any puppy which fitted completely into either the normal or the affected category was regarded as diagnosed, although if the breeder intended to have an affected puppy euthanased a re-test was always advised to guard against a misidentification. In addition, a re-test was always insisted upon for all puppies showing any of the criteria of the doubtful category. Over the full two-and-a-half years of the study only eight puppies (1·4 per cent) required such a re-test, and in all cases the results of the second test were clear-cut. Indeed, all the puppies with initial postprandial bile acid concentrations over 40 μmol/litre proved to be affected on re-testing, whereas all those with concentrations less than this value proved to be negative. However it was considered prudent to continue the policy of re-testing puppies in the doubtful category.

The results of this study demonstrate that the dynamic bile acid test is superior to the fasting ammonia test when screening Irish wolfhound puppies for portosystemic shunts. In the group tested there was no overlap between the normal and the affected puppies in either the postprandial bile acid concentration or the change in concentration with feeding. Even when a generous margin for error of 10 μmol/litre on either side of the apparent cut-off value for the postprandial result was introduced, only a few individuals were recalled for repeat testing, and over 98·5 per cent of puppies were given a definite result on first test. In contrast, there was a substantial overlap in ammonia concentration between the normal and affected groups. In addition, the bile acid test is more convenient to carry out, because there is no need to take young puppies to the laboratory, and samples can be taken by the breeder's veterinary surgeon and sent to the laboratory by post or routine courier.

As the clearest separation between normal and affected puppies was provided by the postprandial bile acid measurement, it was decided after the conclusion of this study to apply a single-sample protocol for routine testing, to reduce both the stress to the puppies and the cost to the breeder. Puppies with postprandial bile acid concentrations over 30 μmol/litre are then required to be re-tested by the dynamic test. This protocol has the advantage that if the puppies are reluctant to eat on the morning designated, a decision to abandon the test and reschedule it is less troublesome than if a set of fasting blood samples has already been collected. This of course necessitates arrangements to be made for a practice representative to witness the feeding of the puppies, either at home or by bringing the litter to the practice premises for the entire test (including feeding) to be performed. The experience with litter MV exemplifies the danger of missing an affected puppy when insufficient attention is paid to feeding, even when no deception is intended. Clear and unambiguous identification of individual puppies is also essential. However, by emphasising these matters to the practices concerned and requiring a signed confirmation that the feeding has been witnessed, no further problems have been experienced.

In this study approximately one puppy in 30 was affected, and one litter in six contained one or more affected puppies. The 277 puppies from addresses in the UK which were tested during 1997 were equivalent to 46 per cent of Kennel Club registrations for the breed in that year, and this incidence is therefore probably reasonably representative of the breed as a whole. A similar incidence has also been reported in Irish wolfhounds in the Netherlands (Meyer and others 1995). Given the high incidence and the availability of a reliable test which can be carried out without transporting puppies to a

laboratory, it seems highly desirable that all Irish wolfhound puppies should be screened for the condition before they are sold on from the breeders' households, and before they have been chosen by a prospective new owner. Testing at six to eight weeks of age should be ideal, although the test remains valid in older animals.

Reports from colleagues in practice indicate that the Irish wolfhound may not be the only breed in the UK in which this problem occurs. Preliminary studies of a small number of Scottish deerhound litters suggest that the incidence of congenital portosystemic shunt may be similar in that breed, and some terrier breeds have also been implicated (M.G. Kerr, unpublished observations). It would therefore seem prudent to consider extending the scope of the testing scheme to include other breeds which may be at risk. However, it has been demonstrated that the test is not valid in Maltese dogs, possibly as a result of the presence of an additional reacting substance in the serum (Tisdall and others 1995), and caution should therefore be exercised when extrapolating the results of this study to breeds other than Irish wolfhound.

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Comparison of cyclosporin A and dexamethasone in the treatment of canine nictitans plasmacytic conjunctivitis

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Thirteen dogs with nictitans plasmocytic conjunctivitis were treated with 2·0 per cent cyclosporin drops in the right eye and with 0·1 per cent dexamethasone ointment in the left eye. The response to both therapies was monitored for six weeks, repeat biopsy specimens were taken, and the time for the clinical signs to recur recorded. Conjunctival cultures were taken before and after both therapies. There were no significant differences between the treatments in the remission of clinical signs, the reduction of inflammatory infiltrate in the biopsy specimens, or the time to recurrence of the condition or its subsequent severity. However, the eyes treated with 0·1 per cent dexamethasone tended to recover more rapidly than the eyes treated with 2·0 per cent cyclosporin, and the eyes treated with 2·0 per cent cyclosporin tended to be protected from a recurrence for longer than the eyes treated with 0·1 per cent dexamethasone.

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CANINE nictitans plasmacytic conjunctivitis is also referred to as plasmoma (Helper 1981), plasma cell infiltrate of the nictitans (Brooks 1991), third eyelid immune-mediated hypertrophy (Cottrell and Peiffer 1989) or chronic inflammation of the external surface of the nictitating membrane (Rubin 1989). The condition has been described most commonly in adult German shepherd dogs, though it can also affect other breeds: Belgian shepherd, borzoi, dobermann, and springer spaniel (Rubin 1989), and collie, greyhound and dachshund (Barnett 1994).

The aetiology of the condition is not entirely clear, but it is suspected that an immune-mediated process induced by ultraviolet light is involved, as in chronic superficial keratitis (Slatter and others 1977, Blogg 1980, Kirschner 1992).

It is a bilateral condition although both eyes may not be affected equally (Barnett 1994). Clinically it is characterised by hyperaemia and thickening of the nictitans conjuntivae, raised pin-head-sized non-pigmented lesions or follicles in the exposed surface of the nictitans and depigmentation of its free border (Teichert 1966, Helper 1989, Rubin 1989, Barnett 1994, Read 1994, 1995). A mucoid or mucopurulent discharge, bulbar conjunctival hyperaemia and follicular conjunctivitis of the bulbar surface of the nictitating membrane have occasionally been described (Blogg 1980, Helper 1981,

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Notes