Cobalamin in dogs

Oliver Waite BVSc MRCVS on the assessment of cobalamin in dogs

Cobalamin (vitamin B12) is increasingly used clinically in small animal medicine. Disorders involving abnormal cobalamin concentrations include intestinal dysbiosis, exocrine pancreatic insufficiency (EPI), chronic gastrointestinal disease and hereditary disorders of cobalamin absorption. Serum cobalamin concentrations are often measured and performed in conjunction with other tests for gastrointestinal (GI) disease including folate (vitamin B9) and canine trypsin-like immunoreactivity (cTLI). The aim of this article is to provide information on assessment of cobalamin and its clinical implications, along with specific examples of derangements in cobalamin metabolism.

WHAT IS COBALAMIN?

Cobalamin is an essential water-soluble vitamin acquired exclusively from foods of animal origin. It is supplemented in commercial diets. The liver plays an important role in storing cobalamin following digestion and absorption.

Cobalamin is bound to dietary protein and once in the stomach, is released by pepsinogen and gastric acid. It is then bound to haptocorrin to protect it from utilisation by proximal intestinal microbiota. In the duodenum, pancreatic proteases cleave cobalamin from haptocorrin and the free cobalamin binds to intrinsic factor (IF). Intrinsic factor (IF) is predominantly synthesised in the exocrine pancreas in dogs, with smaller amounts produced in the stomach.

This protein enables receptor-mediated endocytosis within the ileum; the primary site of canine cobalamin absorption. This ileal receptor complex, called cubum comprises two separate protein subunits, cubilin and amnionless. Receptor-mediated uptake is responsible for almost all cobalamin absorption with < 1 per cent absorbed via passive diffusion across the GI tract. Once within the enterocytes, cobalamin is separated from IF and bound to another transport protein, transcobalamin II, allowing movement into the bloodstream and transport for intra-cellular use of this vitamin.

WHY IS IT IMPORTANT?

All mammals are cobalamin-dependent for two major enzymatic reactions: methionine synthase and methylmalonyl-CoA mutase. Methionine synthase is a vitamin B9 and cobalamin-dependent enzyme responsible for the production of methionine from homocysteine (HCY). Hyperhomocysteinaemia may result in depleted folate concentrations and is associated with cardiovascular disease, birth defects and neoplastic processes in humans.

Methylmalonyl-CoA mutase is responsible for the conversion of methlymalonyl-CoA to succinyl-CoA, an essential molecule of Kerb's cycle. Cobalamin deficiency results in increased methylmalonic acid (MMA) concentrations. Excess MMA is excreted via the urine and can result in MM aciduria. It also inhibits the activity of carbamoyl phosphate synthetase I, responsible for the conversion of ammonia to carbamoyl phosphate within the urea cycle. Hyperammonaemia is a potentially life-threatening consequence recognised in both animals and humans. Alterations in cobalamin concentration can be wide-reaching and manifest in a myriad of clinical presentations.

INVESTIGATING COBALAMIN DERANGEMENTS

Cobalamin measurement

Serum cobalamin concentrations are almost exclusively measured at external reference laboratories. Various methodologies exist but automated chemiluminescent assays remain the most commonly-used method across Europe and North America. Cobalamin is a stable compound in serum for up to five days and thus rapid measurement is not required and samples can easily be taken alongside other haematological analytes required.

The University College Dublin Veterinary Diagnostic Laboratory reports a reference interval between 275 and 1000pg/ml with a lower limit of detection of the assay as <150pg/ml. It is recognised however, that serum concentrations may not truly reflect cobalamin concentrations at the cellular level. Cobalamin concentrations within the low end of the reference interval can be associated with increased MMA concentrations supportive of functional hypocobalaminaemia.

Assessment of cobalamin in human medicine is routinely interpreted together with MMA, homocysteine and holotranscobalamin (representing the biologically active fraction of cobalamin bound to transcobalamin II) concentrations. Measurement of the latter is currently not available for animals and the significance of elevated HCY concentrations is not fully understood in veterinary medicine. Methylmalonic acidaemia may reflect reduced intra-cellular cobalamin concentrations and has been reported in 22 per cent of dogs suggesting the existence of functional hypocobalaminaemia. Caution is, however, advised to ensure alternative causes of increased MMA concentrations such as reduced renal function are considered. Given this, and lack of widely available laboratories offering such measurement coupled with increased costs, investigation of MMA is not routinely performed in small animals.

In line with the result obtained, various terminologies can be used to describe the cobalamin status:

- Cobalamin deficiency – concentrations are inadequate to meet metabolic demand usually indicated by an increased methyl malonic acid concentration;
- Hypocobalaminaemia – concentrations are below the lower limit of the reference interval;
- Normocobalaminaemia – concentrations are within the reference interval;
- Hypercobalaminaemia – concentrations exceed the reference interval.

Haematology Report Slide no:

Hct	0.38	l/l	(0.37 - 0.55)	WBC	46.83	x109/L	(6 - 17)	
Hgb	122.00	g/l	(120 - 180)	NeutA	41.45	x109/L	(3 - 11.5)	(88.50 %)
RBC	5.03	x1012/L	(5.5 - 8.5)					
MCHC	325.00	g/L	(310 - 362)	LymA	2.44	x109/L	(1 - 3.6)	(5.20 %)
MCV	74.50	fL	(60 - 77)	MonoA	2.65	x109/L	(0 - 1.35)	(5.70 %)
MCH	24.30	pg	(19.5 - 25)	EosA	0.18	x109/L	(0 - 1.47)	(0.40 %)
PDW	52.30	%						
MPXI	17.70							
#Retic	190.10	x109/L	(0 - 60)	PT	15.00	secs	(7 - 14)	
Plt	835.00	x109/L	(150 - 500)	PTT	>100	secs	(12 - 25)	
MPV	12.70	fL	(7 - 11)					

Marked leukocytosis with marked mature neutrophilia and mild to moderate monocytosis. Moderate polyhromasia, otherwise normal morphology of red and white blood cells. Marked thrombocytosis. Suggest repeat clotting times

Biochemistry Report

Tot Protein	44.1	g/L	(54 - 71)	Albumin	25.9	g/L	(25 - 38)
Globulin	18.2	g/L	(28 - 42)	Calcium	2.20	mmol/L	(2.3 - 3)
Urea	11.30	mmol/L	(3.6 - 8.6)	Creatinine	51	umol/L	(20 - 120)
LIPASE	75	U/L	(0 - 130)	Amylase	1092	U/L	(400 - 1300)
Glucose	5.81	mmol/L	(3 - 6.5)	Cholesterol	1.85	mmol/L	(3.2 - 6.5)
Triglycerides	0.60	mmol/L	(0.11 - 1.69)	ALP	200	U/L	(0 - 82)
GGT	10.00	U/L	(7 - 16)	Total Bili	0.90	umol/L	(0.9 - 10)
ALT	71	U/L	(0 - 36)	Phosphorus	1.90	mmol/L	(0.8 - 1.8)
GLDH	20	U/L	(0 - 16)	CK	136	U/L	(0 - 122)
AST	32	U/L	(0 - 37)	Sodium	152.0	mmol/L	(137 - 151)
Potassium	3.38	mmol/L	(3.7 - 5.8)	Chloride	115.0	mmol/L	(105 - 117)
CO2	24.6	mmol/L	(17 - 24)	Anion Gap	15.8	mmol/L	(11 - 26)

Urinalysis

Colour:	pale yellow	Epithelial:	-	Urobilinogen:	-
Odour:	normal	RBC:	3+	Bilirubin:	-
Turbidity:	clear	WBC:	1+	Blood:	4+
S.G.:		Protein:	-	Casts:	-
Nitrite:	-	Glucose:	-	Crystals:	-
pH:	6.5	Ketones:	-	Bacteria:	-
Ur Protein:	mg/dL	Ur Creatinine:	mg/dL	Ur Pro Creat Ratio:	
Ur Sodium:	mmol/L	Ur Calcium:	mmol/L	Ur Phosphorus:	mmol/L
Ur Potassium:	mmol/L	Ur Chloride:	mmol/L	Ur Magnesium:	mmol/L

very small sample

Figure 1: Haematological, biochemical and urinalysis results for Example Case I. Courtesy: University College Dublin Veterinary Diagnostic Laboratory

little significance in dogs despite similar elevations in cats being reported in conjunction with hepatic and neoplastic disease. There is now increasing interest in assessing hypercobalaminaemia in dogs but there is limited information thus far.

Gastrointestinal disease

Fundamentally, any case presenting with chronic GI disease should be screened for hypocobalaminaemia. Chronic GI disease is defined as a condition with a history of ≥ 3 weeks. Assessment of cobalamin may aid in localising GI disease as it is almost exclusively absorbed within the ileum. However, normal pancreatic function is also necessary for adequate absorption and cobalamin concentrations are therefore often measured alongside circulating TLI concentrations. Concurrent measurement of folate concentrations may also provide evidence for proximal small intestinal disease.

Folate = 2.05ng/ml (8.2-13.5)
Cobalamin = 187pg/ml (>275)
cTLI – 8.78ng/ml (5-45)

Figure 2: Example Case I, reduced folate and cobalamin concentrations were reported consistent with diffuse intestinal malabsorption, Example Case I. The reference interval TLI concentration rules out exocrine pancreatic insufficiency as a cause of hypocobalaminaemia. Courtesy: University College Dublin Veterinary Diagnostic L.aboratory. [Caption for thumbnail_Image 3.jpg, note the red arrows above are not in the supplied jpg and will have to be positioned by the designer]

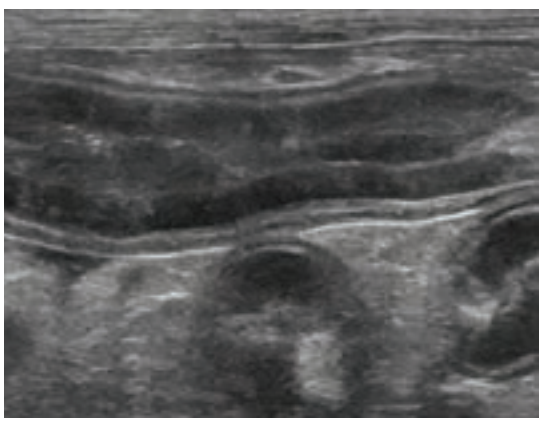


Figure 3: Example Case I, ultrasound examination of the jejunum. Note the evidence of dilated lacteals (red arrows) and a subjective reduction on the definition of intestinal wall layering. Courtesy: University College Dublin Diagnostic Imaging Service.

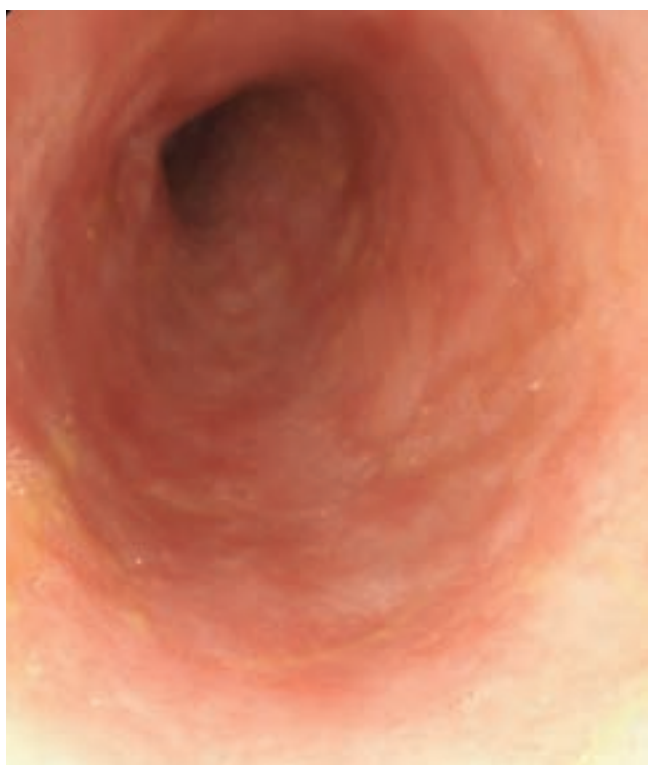


Figure 4: Example Case I, endoscopic examination of the upper gastro-intestinal tract. Note a subjective blunting to the duodenal mucosa. Courtesy: University College Dublin Internal Medicine Service.

Figure 5: Example Case II, note the poor quality and body condition score. Courtesy: University College Dublin Internal Medicine Service.

Chronic enteropathies:
Hypocobalaminaemia develops because of a combination of chronic mucosal inflammation reducing the number and function of cubum receptors together with secondary intestinal dysbiosis preventing adequate cobalamin absorption.Increased MMA concentrations induced by hypocobalaminaemia potentially facilitate further mucosal inflammation and reduce appetite, which is a commonly encountered problem in dogs with chronic enteropathy. Hypocobalaminaemia has been reported in 36 per cent of dogs with chronic gastrointestinal disease. However, only nine per cent of the same cohort exhibited MMA elevations suggesting only a small proportion of similar cases exhibit hypocobalaminaemia at the cellular level.

Exocrine Pancreatic Insufficiency (EPI):
Hypocobalaminaemia is a common finding in dogs diagnosed with EPI. In one study, 82 per cent of dogs with EPI had evidence of reduced cobalamin concentrations. Additionally, hypocobalaminaemia was described as a negative prognostic indicator, especially in the absence of elevated folate concentrations. Absorption of cobalamin is highly dependent on IF produced by the pancreas. Additionally, pancreatic enzymes are required for the cleavage of the initial cobalamin-haptocorrin compound contributing to the development of hypocobalaminaemia. A diagnosis of EPI can be made easily through assessment of cTLI concentrations.

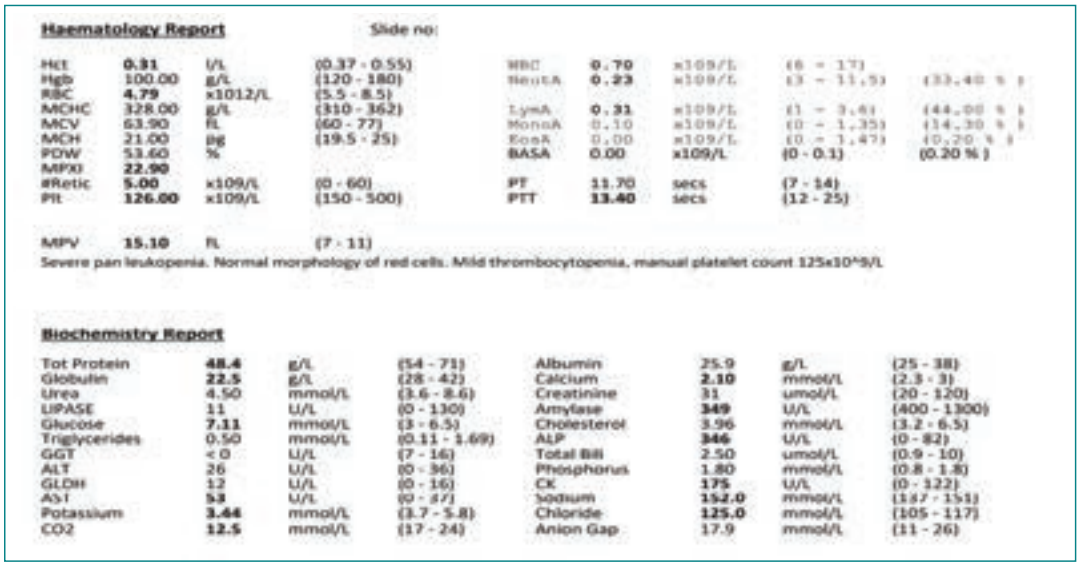
Intestinal dysbiosis:
Intestinal dysbiosis or small intestinal bacterial overgrowth (SIBO) refers to a specific chronic enteropathy presentation with a positive empirical response to anti-microbial therapy and where all other causes have been excluded in a thorough and systemic diagnostic work-up. Hence, SIBO has often been termed antibiotic responsive diarrhoea (ARD). Although the significance of primary SIBO in human medicine is widely debated, it is accepted that secondary cases of SIBO can produce the classical signs associated with ARD. Intestinal dysbiosis promotes hypocobalaminaemia through bacterial and enterocyte competition for nutrients including cobalamin. The prevalence of hypobocobalaminaemia in cases of SIBO is unknown.

Example Case I:
A 12-year-old, male, neutered Irish setter presented following a two-month history of rapid and progressive weight loss, poor appetite and intermittent mixed bowel diarrhoea.
From the clinicopathological data in Figure 1, a severe hypoproteinaemia is appreciated. In order to diagnose this protein-losing enteropathy, evaluation of alternate causes of protein loss is required. Proteinuria was absent meaning renal losses were excluded. Several biochemical abnormalities point in the direction of severe GI disease including: elevated urea but not creatinine concentrations (suggestive of GI haemorrhage), hypocholesterolaemia and a total hypocalcaemia (reflective of hypoalbuminaemia). Normal bile acid stimulation and adrenocorticotropic stimulation tests ruled out hepatic disease and hypoadrenocorticism, confirming the changes as likely due to gastrointestinal disease. Folate, cobalamin and cTLI concentrations were subsequently assessed supporting GI disease.
Final histological diagnosis: Diffuse moderate plasmocytic enteritis with multifocal marked villar blunting and loss.

OTHER DISEASES

Alterations in extra-cellular cobalamin concentrations may result in mild-marked haematological abnormalities including non-regenerative anaemia, and neutropenia. Cobalamin testing may also be performed in selected cases where a suspected genetic cause of malabsorption is present.

Imerslund-Gräsbeck Syndrome (IGS):
Imerslund-Gräsbeck syndrome refers to a rare, autosomal recessive trait in dogs characterised by cobalamin deficiency. It can give rise to stunted growth, ill-thrift, malaise and haematological dyscrazias. To date, the syndrome has been reported in Australian shepherd dogs, beagles, Border collies, Chinese shar peis, giant schnauzers and komondors. A single case report exists detailing the syndrome in a beagle cross dog. Typically, dogs present as juveniles following the depletion of maternally-derived cobalamin stores, and often as stunted with a lacklustre coat and an increased susceptibility to opportunistic infection. The disease is confirmed by documenting cobalamin deficiency, appropriate signalment and genetic testing. Defects of the cubilin sub-protein have been proven in beagles and Border collies. Australian shepherd dogs and giant schnauzers demonstrate an altered amnionless structure. Hence, the genetic tests are breed-specific.
An excellent prognosis exists for dogs diagnosed with IGS, providing life-long cobalamin supplementation is provided.



Haematology Report Slide no:

Hct	0.31	l/L	(0.37 - 0.55)	WBC	0.70	x109/L	(6 - 17)	
Hgb	100.00	g/L	(120 - 180)	NeutA	0.23	x109/L	(3 - 11.5)	(33.40 %)
RBC	4.79	x1012/L	(5.5 - 8.5)					
MCHC	328.00	g/L	(310 - 362)	LymA	0.31	x109/L	(1 - 3.6)	(44.00 %)
MCV	63.90	fL	(60 - 77)	MonoA	0.10	x109/L	(0 - 1.35)	(14.30 %)
MCH	21.00	pg	(19.5 - 25)	EosA	0.00	x109/L	(0 - 1.47)	(0.20 %)
PDW	53.60	%		BASA	0.00	x109/L	(0 - 0.1)	(0.20 %)
MPXI	22.90							
#Retic	5.00	x109/L	(0 - 60)	PT	11.70	secs	(7 - 14)	
Plt	126.00	x109/L	(150 - 500)	PTT	13.40	secs	(12 - 25)	
MPV	15.10	fL	(7 - 11)					

Severe pan leukopenia. Normal morphology of red cells. Mild thrombocytopenia, manual platelet count 125x10^9/L

Biochemistry Report

Tot Protein	48.4	g/L	(54 - 71)	Albumin	25.9	g/L	(25 - 38)
Globulin	22.5	g/L	(28 - 42)	Calcium	2.10	mmol/L	(2.3 - 3)
Urea	4.50	mmol/L	(3.6 - 8.6)	Creatinine	31	umol/L	(20 - 120)
LIPASE	11	U/L	(0 - 130)	Amylase	349	U/L	(400 - 1300)
Glucose	7.11	mmol/L	(3 - 6.5)	Cholesterol	3.96	mmol/L	(3.2 - 6.5)
Triglycerides	0.50	mmol/L	(0.11 - 1.69)	ALP	346	U/L	(0 - 82)
GGT	< 0	U/L	(7 - 16)	Total Bili	2.50	umol/L	(0.9 - 10)
ALT	26	U/L	(0 - 36)	Phosphorus	1.80	mmol/L	(0.8 - 1.8)
GLDH	12	U/L	(0 - 16)	CK	175	U/L	(0 - 122)
AST	53	U/L	(0 - 47)	Sodium	152.0	mmol/L	(147 - 151)
Potassium	3.44	mmol/L	(3.7 - 5.8)	Chloride	125.0	mmol/L	(105 - 117)
CO2	12.5	mmol/L	(17 - 24)	Anion Gap	17.9	mmol/L	(11 - 26)

Figure 6: Example Case II, haematological and biochemical profiles. Courtesy University College Dublin Veterinary Diagnostic Laboratory.

Example Case II:
A 1.5-year-old male entire Border collie presented following chronic history of ill-thrift, hyporexia, and lethargy with a lacklustre coat. A per-acute onset of anorexia, vomiting, mixed bowel diarrhoea and pyrexia resulted in presentation.
From the clinicopathological data (Figure 6), a mild normocytic, normochromic anaemia can be appreciated, consistent with an anaemia of chronic disease and compatible with the chronicity of the presenting history. A mild thrombocytopaenia is present, unlikely to be of clinical significance. A severe leukopenia secondary to a marked neutropoenia suggests a destructive, or non-productive process. The remainder of the biochemical parameters represent non-specific abnormalities which may reflect the acute onset of GI disease the dog presented with.
The mild increase in hepato-cellular enzyme parameters likely reflect recent treatment with glucocorticoids. An adrenocorticotropic stimulation test precluded a diagnosis of hypoadrenocorticism. Urine culture and susceptibility and diagnostic imaging failed to identify an infectious focus.

vetscan

Diagnostic Solutions

• No long-term contract • No quarterly fees • Zoetis support

Contact your Zoetis Account Manager for more information.

Alternatively contact Zoetis, 2nd Floor, Building 10, Cherrywood Business Park, Loughlinstown, Co Dublin, D18 T3Y1. Telephone 01 256 9800 Email Vetscanireland@zoetis.com www.zoetis.ie.

MM-12275 (July 2021)

zoetis

Folate= 16.1 ng/ml (8.2-13.5)
Cobalamin= <150 pg/ml (>275)

Figure 7: Example Case II, cobalamin deficiency detected. Courtesy University College Dublin Veterinary Diagnostic Laboratory

During the hospitalisation period, the dog was treated with intravenous fluid therapy, broad spectrum anti-microbial therapy, anti-emetic therapy and proton pump inhibitors following the history of acute GI disease.
Following the identification of a cobalamin deficiency (Figure 7), genetic testing was performed, confirming the presence of the causative autosomal recessive mutation for IGS. Hydroxycobalamin supplementation was implemented.

TREATMENT

There is rarely a contraindication to supplement cobalamin if reduced concentrations are documented. Different protocols exist for both parenteral and enteral administration of cobalamin (see Table 1). Although parenteral supplementation is usually recommended, oral supplementation can be as successful. An advantage of enteral administration is that repeated and potentially painful injections can be avoided.

CONCLUSION:

Derangements of cobalamin metabolism may be wide-reaching. Investigating and treating cases of hypocobalaminaemia inevitably improves overall patient welfare and in specific cases, may drastically reduce mortality rates. An easy diagnostic sample to take and interpret, cobalamin should be measured in any canine patient with appropriate clinical signs warranting further investigation.

	Enteral supplementation	Parenteral supplementation
Gastro-intestinal disease	50ug/kg SID over 12 weeks	50ug/kg SC/IM injection once weekly for six-weeks with cyanocobalamin One final injection one-month after the initial course Repeat cobalamin assessment monthly thereafter
Imerslund-Gräsbeck syndrome	1000ug SID life-long	Initially 50ug/kg SC/IM injection once weekly for four-six weeks with cyanocobalamin 1000ug every one-two month once levels are controlled life-long

Table 1.

References available on request.

ACKNOWLEDGMENTS

Thank you to the University College Dublin Veterinary Hospital for providing photos and laboratory results displayed. A special thank you to Professor Carmel Mooney for her help and support completing this article.

READER QUESTIONS AND ANSWERS

1 COBALAMIN IS ALSO KNOWN AS:
- **A.** Vitamin B1
- **B.** Vitamin B6
- **C.** Vitamin B9
- **D.** Vitamin B12

2 WHERE IS INTRINSIC FACTOR (IF) PRODUCED IN DOGS?
- **A.** Exocrine pancreas
- **B.** Endocrine pancreas
- **C.** Liver
- **D.** Stomach

3 WHERE IS THE MAJOR SITE OF COBALAMIN ABSORPTION?
- **A.** Stomach
- **B.** Duodenum
- **C.** Jejunum
- **D.** Ileum

4 HYPERCOBALAMINEMIA HAS BEEN ASSOCIATED WITH WHAT IN CATS?
- **A.** Hepatic and neoplastic disease
- **B.** Hepatic and renal disease
- **C.** Neoplastic and heart disease
- **D.** Neoplastic and immune mediated disease

5 ROUGHLY WHAT PERCENTAGE OF DOGS WITH EXOCRINE PANCREATIC INSUFFICIENCY ARE HYPOCOBALAMINAEMIC?
- **A.** 20 per cent
- **B.** 50 per cent
- **C.** 80 per cent
- **D.** 100 per cent

6 WHICH OF THESE BREEDS HAS IMERSLUND-GRÄSBECK SYNDROME NOT BEEN REPORTED IN?
- **A.** Beagle
- **B.** Cocker spaniel
- **C.** Border collie
- **D.** Giant schnauzer

ANSWERS: 1D; 2A; 3D; 4A; 5C; 6B.