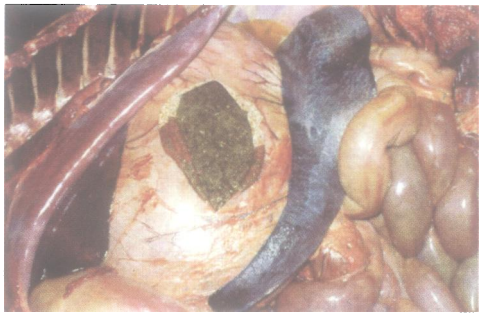

Acute grass sickness can be difficult to distinguish from some forms of surgical colic. In this case of acute grass sickness, there is gastric and small intestinal distension. The peritoneal fluid was clear, deep yellow in colour and had a high protein concentration

Peritoneal fluid analysis for the differentiation of medical and surgical colic in horses

ELSPETH MILNE

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WHEN presented with a case of acute colic, it may be difficult to determine whether immediate surgical intervention is required (for example, to correct an intestinal strangulation), or whether a medical condition, such as acute grass sickness, is present. Clearly, a decision must be made quickly as any delay may have a serious detrimental effect on the outcome. The analysis of peritoneal fluid has been used for many years in the investigation of diseases of the abdominal cavity and, in many acute colic patients, can be helpful in deciding whether an animal has a surgical or medical problem. However, it is important that any findings are considered in the context of the history, clinical examination and results of other ancillary diagnostic procedures. It is vital that other indicators for surgery (eg, unrelenting pain, small intestinal distension, deteriorating cardiovascular function) are appreciated, as these signs often become apparent before changes in the peritoneal fluid are observed. In such cases, there is no need to wait for the results of peritoneal fluid analysis and surgery should be undertaken as a matter of urgency. This article describes how to collect and analyse peritoneal fluid, and highlights some of the changes which may be seen in specific conditions.

FORMATION OF PERITONEAL FLUID

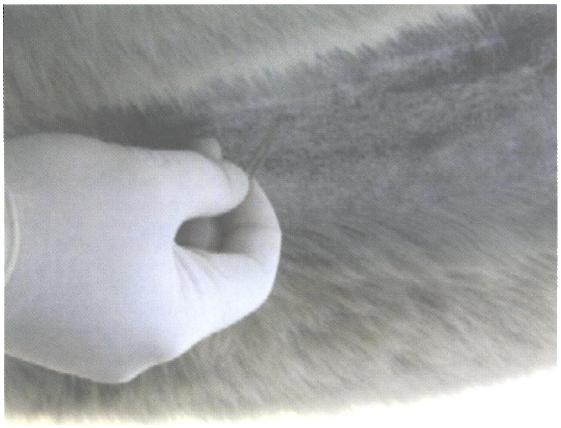
NORMAL FLUID

The peritoneal cavity consists of abdominal and pelvic cavities although there are extra-abdominal extensions, such as the inguinal canal, which are confluent with it. Normal peritoneal fluid is a serous dialysate of blood. It lubricates the abdominal contents, prevents intra-abdominal adhesions and has antibacterial properties. The peritoneum acts as a semipermeable membrane through which small molecules may be exchanged between the blood and abdominal cavity. The fluid is secreted by the peritoneum which consists of a single layer of mesothelial cells overlying loose connective tissue and fat. This serous membrane covers the viscera and lines the peritoneal cavity. Peritoneal fluid is drained by the specialised lymphatic lacunae of the diaphragm to the right thoracic duct and into the systemic circulation.

ABNORMAL FLUID

Abnormal peritoneal fluid may be classified as a transudate, modified transudate or exudate according to its total protein and total nucleated cell count (see table on the facing page), but these categories are not clear-cut. True transudates are low protein, low cellular fluids which are formed as a result of a decrease in colloidal osmotic pressure in the blood (principally due to hypoproteinaemia), increased capillary hydrostatic pressure (congestive cardiac failure or portal hypertension) and lymphatic obstruction. Modified transudates are formed where there is an increase in capillary hydrostatic pressure and also in cases of intra-abdominal neoplasia, uroperitoneum or lymphatic obstruction/rupture. Their characteristics are intermediate between true transudates and exudates. Exudates are formed during inflammatory and ischaemic processes as a result of increased capillary permeability and migration of inflammatory cells across capillary walls. They have a high protein concentration and a high cell count, including neutrophils, macrophages and lymphocytes; in the case of septic peritonitis or gut rupture, bacteria may also be evident.

When vascular compromise of the gastrointestinal tract occurs, an exudate will form and, initially, there is serum exudation and lymph leakage due to gut distension. As the process continues, there is diapedesis of red and white blood cells into the peritoneal fluid which becomes tinged with blood; this process progresses as capillaries break down. The fluid volume increases markedly and becomes dark red/brown in colour and haemolysed with progressive gut wall devitalisation. In medical conditions associated with significant gut distension but no ischaemia, there may be a slight to moderate increase in fluid protein content, total nucleated cell count and volume, or the fluid may be normal.



Peritoneal fluid collection using a hypodermic needle inserted through the ventral abdominal midline

CLASSIFICATION OF PERITONEAL FLUID

Type of fluid	*Total nucleated cell count (x 10^9/litre)*	*Total protein (g/litre)*	*Major underlying process*
Normal	<10	<25	–
Transudate	<5 (usually <1·5)	<25 (usually <15)	Decreased colloidal osmotic pressure Increased capillary hydrostatic pressure
Modified transudate	1·5-10	25-35	Increased capillary hydrostatic pressure
Exudate	>10	>30-35	Increased capillary permeability

From DeHeer and others (2002)

Equipment for peritoneal fluid collection

- Clippers
- Gloves
- Skin antiseptic (eg, povidone-iodine)
- Swabs
- 18 to 20 gauge, 3·8 cm needles and 13 gauge, 10·5 cm jugular catheters
- 'Red top' Vacutainer tubes without anticoagulant
- 'Purple top' Vacutainer or other tubes containing EDTA

PERITONEAL FLUID COLLECTION

METHOD

■ Clip a strip of skin, approximately 8 cm wide, in the ventral midline and prepare the skin using antiseptic swabs.

■ The horse should be well restrained and an assistant should lift the foreleg on the opposite side to the operator. Sedation is rarely required and may be contraindicated. It is also not necessary to use local anaesthesia as fluid collection usually causes less discomfort than administration of the anaesthetic agent.

■ Right-handed operators should stand at the horse's left shoulder while left-handed operators should stand at the animal's right shoulder. The site of needle placement depends on the abdominal shape. In most cases, the lowest part of the abdomen is used. In 'tucked up' horses, a point just behind the xiphoid process often yields fluid.

■ Holding an 18 to 20 gauge, 3·8 cm needle firmly by the hub, with the point upwards, feel for the linea alba and insert the needle quickly through the overlying skin to a depth of approximately 1 cm. Advance the needle slowly, stopping to examine the hub for fluid every few millimetres. If no fluid is obtained, rotate the needle on its own axis and advance it deeper into the abdomen. Allow the fluid to drip into the collection tubes. If little fluid is obtained, give priority to the EDTA tube over the plain tube. The aspiration of fluid using a syringe is not recommended as it increases the likelihood of iatrogenic blood contamination and the negative pressure created may result in occlusion of the end of the needle (eg, with abdominal fat).

■ If no fluid is obtained, it may be necessary to remove the needle and to place a fresh needle cranially or caudally to the original site. In obese animals, there may be several centimetres of internal fat lining the abdomen; in such cases, an intravenous catheter can be used and, once the peritoneal cavity is entered, the stylette can be removed. Some authors advocate the use of a teat cannula, but this causes more discomfort, necessitates the creation of a skin incision and increases the likelihood of blood contamination of the sample. Ultrasound imaging of the abdomen can be helpful in locating a fluid pocket.

■ If blood is obtained, it is important to determine whether clear fluid was collected first followed by blood (suggesting iatrogenic rupture of a small vessel) or whether the blood is uniformly mixed in the sample. Repeat the procedure at a different site to see if the presence of blood is a consistent finding.

■ If intestinal contents are obtained (green/brown with a characteristic smell of fermenting vegetation), it is most likely that the intestine has been accidentally penetrated unless the fluid is bloody or turbid. Repeat the procedure at another site.

■ Sometimes no fluid can be obtained, despite several attempts. This can occur in some normal horses and may be due to an insufficiently long needle (particularly in obese horses), an insufficient volume of fluid at the sites of puncture, distension of the ventral colon resulting in dispersion of fluid away from the ventral midline or a reduction in fluid volume as a result of dehydration.

COMPLICATIONS

Complications associated with peritoneal fluid taps are uncommon, but can include penetration of the gut resulting in peritonitis, subcutaneous abscessation and, very rarely, tearing of the gut or spleen. In foals, there is a greater risk of accidentally tearing the gut. This may be avoided by using a blunt cannula and ultrasound to help locate the fluid. Antibiotic cover is usually not required, although it may be advisable if gut penetration has occurred.

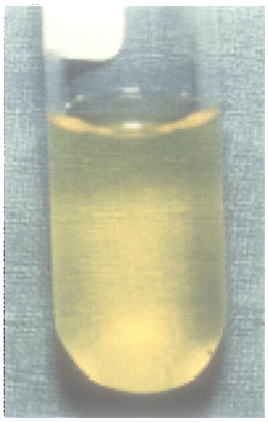
PERITONEAL FLUID ANALYSIS

The appearance, and biochemical and cellular constituents of peritoneal fluid reflect the state of the mesothelial surfaces and the organs they cover. The smaller constituents, such as electrolytes, creatinine and urea, reflect local and/or systemic events. In the field, only the gross appearance and, possibly, the protein content and/or specific gravity of peritoneal fluid can be quickly assessed.

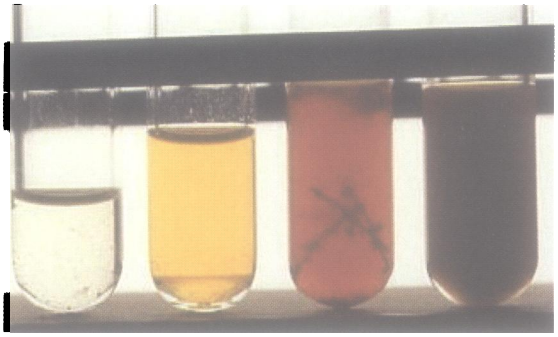
GROSS EXAMINATION

Volume

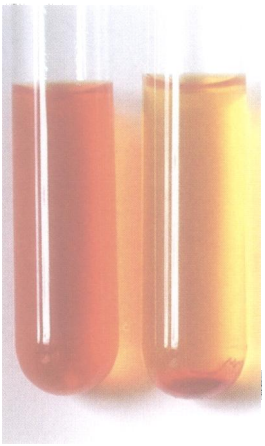
A subjective assessment of peritoneal fluid volume can be made at the time of sample collection based on ease of collection and flow rate. However, the fluid may be 'pocketed' within the abdominal cavity, and a low flow rate does not always correlate with a low volume.



Normal peritoneal fluid



Gross appearance of peritoneal fluid. The sample on the far left is a low protein transudate. The other samples have been collected from cases of (from left to right) equine grass sickness, acute ischaemia with gut rupture and large colon torsion



Normal peritoneal fluid with iatrogenic blood contamination before centrifugation (left) and after centrifugation (right). The pre-centrifugation sample is similar to that seen in early ischaemia (as pictured below left), but note the presence of the red blood cell pellet and clear supernatant after centrifugation

Appearance

Normal fluid is clear, sometimes with a few small white flocculi, and pale yellow in colour. It should not clot in the plain tube (which would suggest the presence of fibrin).

Deep yellow, clear to slightly turbid peritoneal fluid may suggest an uncomplicated medical colic such as colonic impaction or grass sickness where the deep colour may be caused by dehydration and/or hyperbilirubinaemia. Hyperbilirubinaemia is frequently present in horses which have been anorexic for more than two to three days.

Pink, orange or red fluid will be seen in cases of haemolysis or haemorrhage, but iatrogenic contamination must be distinguished from intra-abdominal haemorrhage. If there is blood contamination during collection from a case with no vascular compromise, the fluid may appear pink or orange but, when centrifuged or allowed to stand, the red blood cells form a small pellet and the supernatant will be clear yellow. Early vascular compromise of the gut may also result in homogeneously orange fluid, which is sometimes turbid; however, following centrifugation, no red blood cell pellet (or a small pellet) would be present, and the supernatant would be orange in colour. This is an important distinction and failure to recognise it can result in blood contamination being misinterpreted as bowel ischaemia. In the case of an accidental splenic tap, the fluid will be dark red, with a packed cell volume higher than that of the peripheral circulation. Following intra-abdominal haemorrhage, the fluid will be red but, unless the haemorrhage is very recent, the blood will not clot. On centrifugation, there may be a small- to medium-sized red blood cell pellet and a red haemolysed supernatant.

Turbid, bloody to brown fluid occurs later in cases of ischaemia, with the additional presence of ingesta if gastric or intestinal rupture has occurred.

In cases of peritonitis, in the absence of ischaemia, the fluid will be turbid, yellow to brown and have a thick buffy coat (white blood cell layer) after settling or centrifugation.

Peritoneal fluid from an early case of gut ischaemia

Smell

Gross examination of peritoneal fluid should also include assessment of the smell; the presence of gut contents, bacteria or urine may be suspected from the smell.

PROTEIN CONTENT AND SPECIFIC GRAVITY

Protein concentration and specific gravity are closely correlated. In the field, one or both of these parameters can be assessed using a hand-held refractometer. (The specific gravity pad on urine dipsticks is not reliable.) Increased protein and specific gravity values are expected in animals with peritonitis and are often seen in cases of surgical colic which are accompanied by significant inflammation or vascular compromise of the gut. These values are usually normal in other medical colic cases such as colonic impactions (see section on specific conditions for exceptions).

OTHER BIOCHEMICAL CONSTITUENTS

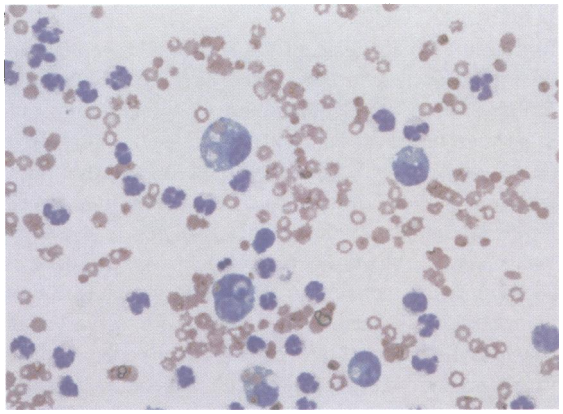
Biochemical constituents, other than protein, are of less value in distinguishing between medical and surgical colic cases, and are not usually measured. However, concentrations of peritoneal fluid phosphate, the intestinal isoenzyme of alkaline phosphatase, fibrinogen and lactate may potentially be helpful. In cases where rupture of the bladder is suspected, concurrent measurement of serum and peritoneal fluid creatinine may be performed; uroperitoneum results in a peritoneal fluid creatinine concentration at least twice that of serum. Urea is less useful for this purpose as it more rapidly equilibrates between peritoneal fluid and blood.

TOTAL NUCLEATED CELL COUNT

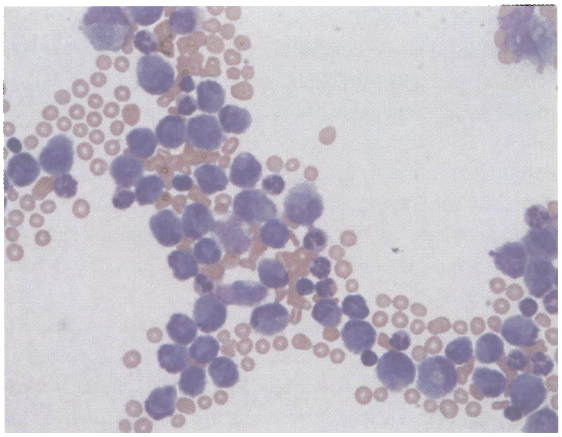
The total nucleated cell count is of value if time and facilities allow, and is required for the correct interpretation of cytology. It can be performed manually using a haemocytometer, or using an automated analyser. The total nucleated cell count will increase in cases of vascular compromise, peritonitis and other inflammatory lesions, but not markedly in most other medical conditions. The total nucleated cell count often parallels the values for protein content and specific gravity.

CYTOLOGY

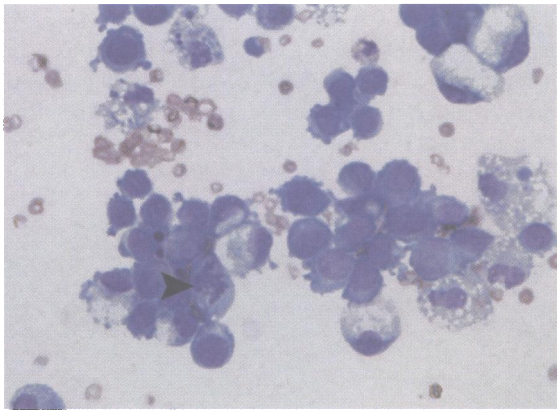
Cytological examination is rarely performed in an emergency situation although it may provide useful retrospective information. Direct smears can be made from the fluid collected into EDTA tubes using blood smear techniques. If the fluid is not highly cellular, prior centrifugation and examination of the deposit will aid interpretation. Smears should be stained with a Romanowsky stain, such as Diff-Quik or Leishman's, and assessed for the presence and number of (few, moderate or many) red blood cells, inflammatory cells (macrophages, lymphocytes, plasma cells, neutrophils and eosinophils) and activated mesothe-



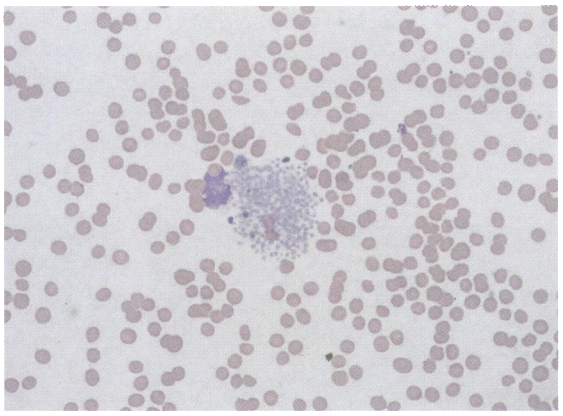
Cytology of peritoneal fluid from a case of small intestinal ischaemia. Neutrophils predominate, with smaller numbers of macrophages and red blood cells. There are two macrophages in the centre which have phagocytosed red and white blood cells. Leishman's stain, magnification x 600



Cytology of peritoneal fluid from a case of chronic peritonitis. Macrophages predominate, with smaller numbers of neutrophils. Leishman's stain, magnification x 600



Cytology of peritoneal fluid from a case of alimentary lymphoma. Note the large number of lymphocytes, one of which is undergoing mitosis (arrowhead). Leishman's stain, magnification x 600



Cytology of peritoneal fluid showing iatrogenic blood contamination at sampling. Many red blood cells and a large group of platelets are present. Leishman's stain, magnification x 600

lial cells (which take on a phagocytic function). The proportion of each inflammatory cell type present helps to classify inflammatory processes into acute, subacute and chronic, and may help to identify a specific aetiology.

The presence of bacteria, neoplastic cells, macrophages which are showing erythrophagocytosis, and other material such as plant fragments or crystals from the urinary tract should be noted. The identification of erythrophagocytic macrophages is useful in determining whether any blood in the sample was present in vivo (ie, blood in the peritoneal fluid prior to collection), as macrophages will phagocytose free red blood cells in vivo. After a few days, the haemoglobin in the engulfed red blood cells will be converted to haemosiderin which appears as dark blue/black granules in the macrophage cytoplasm; this suggests previous haemorrhage. Platelets indicate very recent (often iatrogenic) haemorrhage.

BACTERIOLOGY

A sample of fluid collected into a plain tube may be submitted for bacteriology if peritonitis is suspected (EDTA will inhibit the growth of some bacteria). This may provide invaluable information in cases of peritonitis, but cannot distinguish between acute medical and surgical colic cases due to the time required for culture. Although many samples obtained from animals with peritonitis (not associated with surgery or intestinal rupture) are sterile, *Streptococcus zooepidemicus*, *Bacteroides* species and *Actinobacillus equuli* have been isolated.

PERITONEAL FLUID CHANGES IN SPECIFIC CONDITIONS

There have been numerous studies on the predictive value of peritoneal fluid analysis in cases of medical and surgical colic and these have produced differing results. Many authors agree, however, that, of the readily available tests on peritoneal fluid, gross appearance is the single most valuable indicator of the need for surgery. A combination of gross appearance, protein concentration and/or specific gravity improves the predictive value. The normal constituents and a guide to the findings in some disease categories are shown in the table below.

PERITONEAL FLUID FINDINGS IN CASES OF EQUINE COLIC

	Appearance	*Total nucleated cell count (x 10^9/litre)*	*Total protein (g/litre)*	*Specific gravity*
Normal	Pale yellow, clear	0·5-5·0	5-15	1·000-1·015
Medical colic	Yellow, clear to slightly turbid	5·0-15·0	16-25	1·016-1·020
Surgical colic	Yellow/orange to pink/red/brown, turbid	>15·0	>26	>1·021
Acute grass sickness	Deep yellow, clear to slightly turbid	1·1-40	14-62	1·015-1·041

NB These values are for guidance only and there is often an overlap between categories

MEDICAL COLIC

In uncomplicated conditions, such as primary pelvic flexure impaction, few changes are expected in the peritoneal fluid, with possibly only a slight increase in protein content and specific gravity, and a deeper yellow colour than normal. In cases of acute and subacute grass sickness, the appearance of the fluid is similar to other medical colics, but the protein concentration and specific gravity are high as in some cases of surgical colic (Milne and others 1990).

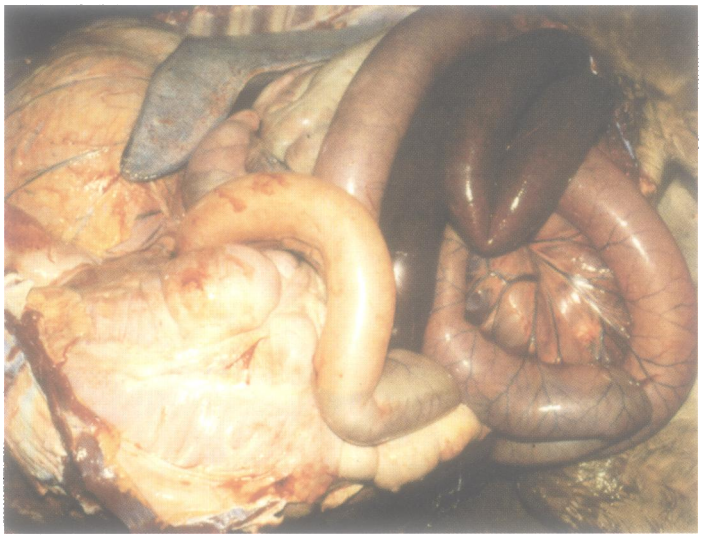
Acute parasitic conditions, such as mass transabdominal migration of large strongyles, may be suspected if more than a few eosinophils are found on peritoneal fluid cytology. However, in many cases of parasite-associated colic, the peritoneal fluid is either normal, similar to that seen in uncomplicated medical colic, or indicative only of a non-specific peritonitis.

Some horses with acute enteritis may present with colic prior to the onset of diarrhoea. In such cases, the changes in peritoneal fluid are minimal or reflect early peritonitis. In animals suffering from sand impaction, the findings usually suggest a medical colic, but devitalisation of the gut wall may occur in some patients, with evidence of an acute inflammatory response on peritoneal fluid analysis.

SURGICAL COLIC

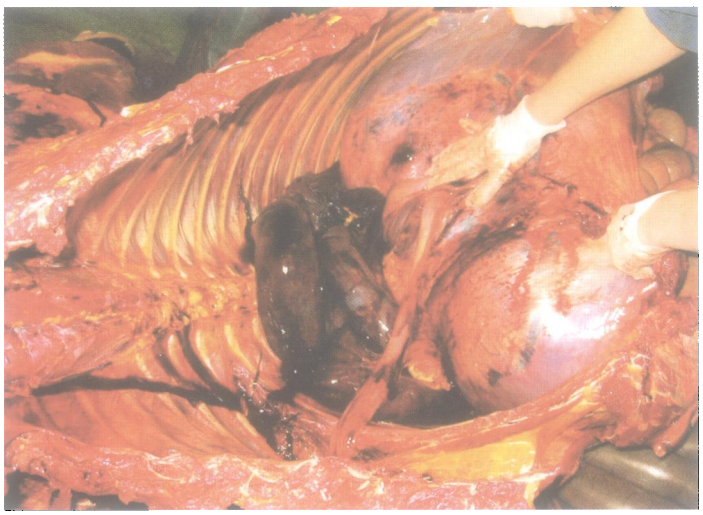
In cases of strangulation of the small or large intestine, or *Strongylus vulgaris*-associated thromboembolic colic resulting in vascular compromise of a segment of gut, an increase in peritoneal fluid volume and a progressive change to haemolysed and then red/brown, turbid fluid, with a high total protein and cell count may occur. However, while peritoneal fluid analysis is useful in many cases to broadly classify whether or not there is compromised circulation to the gut, these categories do not necessarily equate with the need for surgery. For instance, fluid suggestive of a medical colic without vascular compromise might occur in conditions which require surgical intervention (eg, impaction of the ileum). In addition, some colics associated with gut ischaemia may lead to the sequestration of abnormal peritoneal fluid in a site which is not sampled during routine abdominocentesis, resulting in the collection of a sample which appears normal or consistent with a medical colic. Conditions which may result in such a scenario include certain cases of herniation (eg, through the epiploic foramen, and inguinal or diaphragmatic hernias) and intussusceptions, where the compromised gut (intussusceptum) is contained within the intussuscipiens.

Sand impaction of the colon. In this case, the gut wall is becoming devitalised. Peritoneal fluid analysis showed a slightly turbid, orange exudate suggesting ischaemia



Herniation of the small intestine through a mesenteric tear. The peritoneal fluid was dark red, turbid and did not clear on centrifugation

Torsion of the dorsal and ventral large colon cranial to the caecum. The peritoneal fluid was a turbid, brown exudate



Herniation of a segment of small intestine through a diaphragmatic tear. The ischaemic gut was sequestered in the thorax so the peritoneal fluid was unremarkable

OTHER CONDITIONS

Horses with intra-abdominal neoplasia rarely present with acute colic unless there is a complicating factor (eg, strangulation of the small intestine by a pedunculated lipoma or rupture of the gut associated with an intestinal neoplasm), in which case the peritoneal fluid analysis results will be dominated by the superimposed acute process. Most animals with intra-abdominal neoplasia do not shed neoplastic cells into the peritoneal fluid. However, neoplastic cells are occasionally detected in cases of, for example, lymphoma, intestinal adenocarcinoma, gastric squamous cell carcinoma and melanoma.

Peritoneal fluid analysis was found to be normal in mares studied from 10 days prepartum to seven days postpartum, suggesting that pregnancy and parturition should not be a confusing factor and that repeated taps over this timescale should not alter the peritoneal fluid characteristics (Van Hoogmoed and others 1996). Clearly, fluid collected after abdominal surgery will reflect varying degrees of inflammation and haemorrhage.

AN AID TO DECISION MAKING

Peritoneal fluid analysis is a useful ancillary diagnostic procedure to help determine the need for surgery in cases of acute equine colic. Of the parameters which can be quickly assessed in the field, gross appearance is the most valuable indicator of the presence of devitalised gut and the need for immediate surgery, especially if specific gravity and/or protein concentration are measured concurrently. However, the limitations of this type of analysis must be appreciated, and the information it provides should complement rather than replace the evidence provided by a detailed clinical examination. It is also important to note that other indicators for surgery may become apparent before changes in the peritoneal fluid occur and, in such cases, surgery should be undertaken immediately without waiting for the peritoneal fluid to appear abnormal.

Acknowledgements

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