

Farm animal diagnostic tools

Karin Mueller

*MVSc DCHP DipECBHM MRCVS, RCVS & European Specialist Cattle / Bovine Health
Senior Lecturer Animal husbandry & Reproduction
Institute of Veterinary Science, University of Liverpool, Leahurst Campus, Chester High Road,
Neston CH64 7TE*

Several diagnostic tools are available to compliment physical examination of bovine patients, thereby narrowing down the list of differential diagnoses or shifting a tentative diagnosis to a definite one. A lot of information can readily be obtained from cow-side evaluation of samples, backed up by additional laboratory examination where indicated. This article summarises techniques and interpretation of useful diagnostic aids, including abdominocentesis, rumen fluid analysis, liver biopsy, cerebro-spinal fluid analysis, and urinalysis.

ABDOMINO-CENTESIS

Indication

To rule in or out peritonitis, internal haemorrhage, rupture of a portion of the gastro-intestinal tract, uroperitoneum.

Preparation & Equipment

The site is clipped and surgically prepared. Ultrasound-guidance is useful, but not essential. 18-20 gauge hypodermic needle of 5 cm (2") length for dairy cattle, 6.25 – 7.5 cm (2.5 – 3") for high BCS beef cows or bulls. 5 or 10 ml syringe. Small EDTA (cytology & protein) and plain (culture) sample container or vacutainer.

Restraint

Standing, with manual restraint for calves or crush / gate / AI stall for older cattle. Sedation and local anaesthetic is typically not required. A pain response or resentment to needle penetration of skin and peritoneum is likely – anti-kick measures are useful (for example tail jack, anti-kick bar, tying out ipsilateral hindleg), as well as careful positioning of oneself.

Technical description

In ruminants, most suitable sites for collection are: (1) left paramedian, one hand-width behind elbow of animal; (2) left or right paramedian, just in front of udder (taking care to avoid milk vein). The needle is inserted perpendicular to the skin and advanced until fluid appears. If fluid is not forthcoming, suction using a syringe may be tried or the needle slightly repositioned. Choice of site (1) or (2) depends on whether a localised cranial or caudal problem is suspected. For generalised problems, either site is suitable. However, the omentum is very effective in sectioning off fluid, and sampling at two or three sites or depths may be necessary to obtain a sample.

Potential complications

Overall rare and of limited severity. Puncture of viscera is possible, but a needlepoint defect typically seals quickly. Therefore, unless the animal moved markedly during sampling, potentially causing laceration of a structure, no action is required.

Where clinical impression suggests abnormal fluid is likely present, but a sample cannot be obtained, the clinical impression should be trusted.

Analysis

Normal peritoneal fluid is straw-coloured and clear. In late pregnancy, peritoneal fluid is often copious, slightly cloudy, and may clot. Visually detectable abnormalities include: volume greater than 10 ml indicating pathology (or late pregnancy); fibrin or clotting indicating inflammation; ingesta or green discoloration indicating rupture or needle penetration of GI tract; red or pink discoloration or blood (with or without blood clots) indicating haemorrhage or puncture of vessel during collection; reddish-brown fluid indicating gut necrosis. The sample is shaken vigorously to check for elevated protein levels – if present, a stable head foam will form (note: a second aliquot should be used for this, because it will render the sample unsuitable for running through an analyser).

Follow-up analysis in the practice includes: protein concentration (refractometer or analyser), cell count (smear stained with Diff-Quik), bacterial contents (examination of smear and culture). Normal protein levels are <30 g/l. Normal nucleated cell count is <6,000 / μ l (PMNs / granulocytes). Degeneration of PMNs indicates infection.

A creatinine concentration of 1.5 – 2 times or more the animal's serum level is indicative of an uroperitoneum.

RUMENOCENTESIS

Indication

Diagnosis and monitoring of SARA (sub-acute rumen acidosis) on a herd level. Diagnosis of rumen indigestion or acute carbohydrate overload in individual cattle.

Preparation & Equipment

For routine monitoring, ideal sampling time is 3 hours after morning feeding. The site is clipped and surgically prepared. 18-20 gauge hypodermic needle of 5 cm (2") length for dairy cattle, 7.5 cm (3") for high BCS beef cows. 20 ml syringe. Universal plain containers, pH indicator paper (ideally with 0.2 unit increments) or pH meter, 0.03% methylene blue.

Restraint

Standing, with manual restraint for calves or crush / gate / AI stall for older cattle. Sedation and local is not required. A pain response or resentment to needle penetration of skin and peritoneum is likely – anti-kick measures are useful (for example tail jack, anti-kick bar, tying out ipsilateral hindleg), as well as careful positioning of oneself.

Technical description

The needle is inserted perpendicular to the skin and advance fully up to the hub – either about a hand-width behind the last rib in the lower third of the flank, or in the left ventral abdomen behind the xiphoid cartilage. Suction is applied with the syringe and 10-20 ml of fluid collected.

Potential complications

Localised peritonitis is possible, but complications are overall rare and of limited severity.

Analysis

- 1) Colour: green with grass diets, brownish-yellow with straw or maize silage diets.
Abnormal colours include: milky-grey indicating acidosis; light brown indicating simple inactivity; dark brown to black indicating decomposition of rumen contents
- 2) Odour: Normally aromatic, partially resembling diet components.

Abnormal odours include: acidic indicating carbohydrate overload or abomasal reflux; stale indicating simple inactivity; ammoniac indicating urea poisoning; repulsively musty or foul indicating protein decomposition.

- 3) pH: Normally between 5.5 – 7.0, however this is diet dependant. Animals on a roughage-rich diet will show a reduction to about 6.2 between 3 – 4 hours after feeding. Animals on a concentrate-rich diet will show a reduction to 5.5 about 3 hours after feeding. An apparently normal pH should also be interpreted in light of case history: for example, a pH of 6 in an animal inappetent for the last 24 hours is abnormally low.
Abnormal pH values include: alkaline (7.5 – 8.5) indicating starvation for >24 hours, simple inactivity of the rumen flora, urea poisoning, protein decomposition; acidic (<5.5) indicating carbohydrate overload or reflux of abomasal fluid.
- 4) Sediment activity time, based on observation of fresh fluid in a cylindrical container. Normally, fine food particles and protozoa will sink, while larger and fibrous material is carried to the surface, and this process takes 4 – 8 minutes to complete. A distinct greyish layer at the bottom of the tube indicates large protozoa are present and the sample is active. Abnormalities include: rapid sedimentation with slow or absent flotation indicating starvation, acidosis or simple inactivity; rapid flotation or particles remaining in suspension indicating a concentrate-rich diet or protein decomposition.
- 5) Methylene blue reduction test: 20 ml of warm fresh rumen fluid is added to 1 ml of 0.03% methylene blue. A second tube or container is filled with plain rumen fluid for colour comparison and the time measured until the stained sample is decoloured again. Normally, this takes 1 minute on a pure concentrate diet, 3 minutes on a mixed diet, and 3 – 6 minutes on a hay diet.
Decolouration taking > 6 minutes is abnormal and indicates poor activity of the microflora, for example because of acidosis, starvation, or a ration deficient of effective fibre.
- 6) Follow-up examination in the practice includes:
 - a. Microscopic examination for protozoa. One drop of rumen fluid is placed onto a slide and a coverslip added, then examined with 80 – 100 magnification (10x objective). Large, medium and small protozoa should be present and, if the sample has been kept at body temperature and using a warm slide, moving about actively. In digestive disorders, the large protozoa are the first to disappear.
 - b. Gram stain of an air-dried smear. In normal fluid, gram -ve bacteria prevail, and there is a large variety of shapes and sizes. Uniformity of bacteria indicates a diet rich in starches or a digestive disorder. In cases with acidosis, gram +ve bacteria prevail and rod-shaped lactobacilli may prevail.

LIVER BIOPSY

Indication

Copper status on a group level; pathology in individual animals (for example, fatty liver infiltration, neoplasia, photosensitisation).

Preparation & Equipment

The animal should not be starved: a full rumen will aid the procedure by pushing the liver against the right abdominal wall. Consider assessing clotting ability: a blood sample is drawn into a plain glass vacutainer. In a normal animal, the sample will clot within 5 minutes. The site is clipped and surgically prepared. Ultrasound guidance if desired.

5 ml syringe and 23g 1” needle for local anaesthetic; scalpel blade; Tru-Cut®, spring-loaded or other liver biopsy needle / trochar (14 gauge or 4 mm OD, 25 – 30 cm long); skin staples or 5 metric suture material on cutting needle plus needle holder and rat-tooth forceps.

De-ionised sterile water or saline and plain sample pots for copper analysis; pots with 10% formalin for histology. Rapid instrument disinfectant solution and sterile saline, if sampling several animals.

Restraint

Standing in a crush or AI stall. Sedation is not required. 5 ml of local anaesthetic is applied subcutaneously and into the intercostal muscles.

Technical description

The insertion point is in the right 11th intercostal space (i.e. second but last space) on a line drawn between the tuber coxae and the shoulder joint (or about 15 cm ventral to the lumbar transverse processes). A stab incision is made into the skin and into part of the intercostal muscle. With the wrist resting on the animal, the biopsy needle is advanced either perpendicular to the skin and aiming straight across the abdomen, or pointing cranio-ventrally towards the contra-lateral elbow. Appreciating the tissue layers during steady advancement is important: these are intercostal muscle, pleura (resistance followed by a ‘pop’), diaphragm (slight ‘pop’; needle will move strongly with each breath once in diaphragm) and liver capsule (third ‘pop’ about 1 – 2 cm after diaphragm penetration). In adults, the liver is typically reached at about 20 cm depth. Once the liver capsule has been penetrated, the needle is pushed into the liver tissue for a couple of centimetres (slightly gritty feel to it, like pushing needle through sand) and the mechanism of the needle activated to take the sample. A staple or suture is placed into the skin incision.

Potential complications

Main risk factors include penetration of the gall bladder and haemorrhage from penetrating a large hepatic vessel. Peritonitis is very rare. Animals occasionally exhibit dyspnoea after the procedure due to a pneumothorax. Because of the complete mediastinum in cattle, this resolves quickly (within 20 minutes).

Analysis

For copper, blood is washed off the sample with de-ionised sterile water or saline, before placing into a plain sampling pot. For histology, the sample is placed into 10% formalin. Normal liver copper levels are 300 – 8000 µmol/kg dry matter (or 95 – 2000 µmol/kg wet matter). For herd monitoring, 5 animals are sampled and the mean taken.

CEREBRO-SPINAL FLUID (CSF; lumbo-sacral)

Indication

Neurological signs, or suspected trauma and inflammatory or infectious conditions affecting the CNS or spinal cord.

Preparation & Equipment

The area over the lumbo-sacral space is clipped and surgically prepared.

Hypodermic needle 20-21g x 2” for calves, or spinal needle 18 g x 3.5” for adults. 5 – 10 ml syringe, EDTA and plain sample pots.

Restraint

Calves: Sternal recumbency with an assistant sitting astride over the withers facing backwards, and pulling the calf's backlegs forward along each side of the body.

Older cattle: crush with the width reduced if necessary (e.g. old tyres) to prevent sideways movements.

Local anaesthetic (2 – 3 ml calves, 5 ml adults) is injected subcutaneously and into the muscle.

Technical description

The lumbo-sacral space can be felt just caudal (1-2 fingerwidths) to an imaginary line connecting the tuber coxae. When placing the index finger onto the dorsal spinous processes just cranial to this line, and then drawing it caudally with some pressure, the finger will 'fall' into the space.

The surgeon stands or kneels behind the patient. The collection needle is introduced dead midline into the space at an angle of 75° to the skin (i.e. slightly off perpendicular with the hub angled caudally). With the wrist resting on the animal, the needle is steadily advanced, trying to appreciate the layers it penetrates namely: skin, subcutaneous tissue and muscle, interarcuate ligament and ligamentum flavum (slight 'pop') followed by entering the epidural space, then dura mater (another slight 'pop') to enter the subarachnoid space. The 'hanging drop' technique is useful to ascertain when the epidural space has been reached. After entering the subarachnoid space, CSF will typically well up after 10 seconds, and can be collected free-flow or with a syringe (break seal of syringe plunger before connecting).

Potential complications

Needle advanced too deeply, resulting in a pain response to penetration of the conus medullaris. Lateral deviation of the needle resulting in haemodilution of the sample. Introduction of infection into the spinal canal. If two attempts, using a fresh needle, fail, the procedure should be abandoned.

Analysis

An aliquote is placed into EDTA for cytology, and another into a sterile plain pot for culture.

On-farm assessment includes: orange discolouration indicating previous haemorrhage into the spinal canal; precipitation after vigorous shaking of the sample indicating raised protein levels (note: a second aliquot should be used for this, because it will render the sample unsuitable for running through an analyser).

Follow-up analysis in the practice includes: protein concentration (refractometer or analyser), cell count (smear stained with Diff-Quik), bacterial contents (examination of smear and culture).

Normal values are: protein <0.4 g/l; nucleated cell count <0.0 – 8.0 /µl; RBC nil; bacteria nil.

URINALYSIS

Indication

Suspected urinary tract disease, but also metabolic disease like ketosis and to monitor effectiveness of DCAB ration

Preparation & Equipment

In females, the perineal area is dry-wiped for normal urinalysis. If culture is performed on a voided sample, disinfection of the perineal area is recommended. In males, long preputial hair is trimmed with scissors.

Sterile and normal sample pots. Mare urinary catheter and KY-Jelly, unless voided samples is sufficient.

Restraint

Manual restraint for calves, crush/ gate / AI stall for adults.

Technical description

Female: For a voided sample, the perineal area is stroked without holding the tail or disturbing the cow in any other way. For catheterisation, the urethral opening is located on the ventral vaginal floor about 8 – 10 cm cranial to the vulva. A sterile mare urinary catheter, lubricated with KY Jelly, is gently inserted and advanced, taking care not to become lodged in the sub-urethral diverticulum.

Male: Gentle stroking of the perineal area or sigmoid flexure is successful in some males. Stimulation techniques used in the stallion (e.g. whistling, running water, shaking up bedding) are rarely successful in the bull. Catheterisation is extremely difficult in the bull because of the sigmoid flexure and sub-urethral diverticulum.

Potential complications

Introduction of infection during catheterisation.

Analysis

A human urine dipstick is useful for on-farm assessment. However, it is not accurate for specific gravity and this should be determined with a refractometer. For DCAB herd monitoring, pH indicator paper or a pH metre is often more useful than a dipstick because of their finer graduation.

Normal parameters are: Alkaline with pH 7.0 to 8.0; specific gravity 1.020 to 1.040 (may be below 1.020 in high-producing dairy cows); low concentrations of Na, Cl, P, Ca, Mg; high concentrations of K; trace of protein; small amount of ketones. Crystalluria is not significant unless a very large amount is present; calcium-carbonate and triple phosphate crystals are common in normal urine.

Possible changes and their causes include:

- pH can rise with some bacterial infections, but usually falls with urinary tract disease
Cattle may show paradoxical aciduria, where urine pH is acidic despite the animal suffering metabolic alkalosis.
- Specific gravity < 1.020 and no change despite water deprivation or administering large quantity of water indicates renal insufficiency
- Proteinuria indicated renal compromise. It can have physiological causes (stress like recent transport, fever, heavy lactation). May get false positive with human dipsticks in alkaline urine.
- Bacteria – Direct counts: < 10,000 bacteria/ml = contamination; 10,000 to 100,000 = questionable; > 100,000 = infection
- Glycosuria: Enterotoxaemia due to *Cl. perfringens* type D; diabetes (very rare in cattle)
- Haematuria: Blood clots or discoloration or brown sediment; microscopy confirms presence of RBC; usually also positive for haemoglobin on the dipstick. Rule out contamination from reproductive tract in cows, especially if recently calved.
- Haemoglobinuria: false positive result possible caused by lysed RBC (use microscopy to ascertain).
- Myoglobinuria: due to myopathies; differentiate from haemoglobin by spectrography and absence / presence of raised muscle enzymes