Cow-Side test: What is useful and cost-effective in cattle practice?

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INTRODUCTION

On-farm ancillary exams have been considered as needless in cattle practice. For a long time most of these exams could only be performed by laboratories. Only few analyses could be done on the field, but with inaccurate, slow or expensive results. Nowadays, new devices appeared on the market, with greater accuracy for lower prices. The goal of these tests is not only to provide a better diagnosis, but also to improve communication with the owner, and also to give a quick therapeutic answer or a prognosis.

The ancillary exams have to be profitable for both the owner and the practitioner. It can be done at an individual or herd level to demonstrate the subclinical diseases. The benefits of field-tests are essentially saving time, and for some assays, saving money as well, with relatively accurate analyses. In order to adapt to the situation, it is advised to know the sensitivity and specificity of the tests before doing the analysis. In some cases it is necessary to have a better sensitivity or specificity to adapt to goals: avoiding false-positive or false-negative results. A good specificity of a test is required while looking for a diagnosis, as for a screening approach, a good sensitivity is preferred.

Anyway, the use of field tests must be thought carefully and realised only after complete clinical examination of the patient. As it is previously mentioned, the ancillary exams are an important communication tool with the client, and a starting point to a dialogue to convince him to the implementation of appropriate corrective measures in the herd.

Here are only presented the different field tests that can be done within 15 minutes, beside the animals, in the barn. Tests are explained in the well-known order (as a disease will act): inflammatory, metabolic, immunological response and the potential presence of the infectious agent.

INFLAMMATORY CONSEQUENCES

Inflammatory process can occur in diseases associated or not with an infectious agent. It results in an increase of inflammation proteins (haptoglobin, ceruloplasmin, fibrinogen, gamma-globulins) and modifications of the blood formula (neutropenia, in the acute phase).

Among these positive markers of inflammation, gamma-globulins and fibrinogen can be estimated on the field.

a. Coagulation with glutaraldehyde (several manufacturers in Europe: Graeub, NBVC,…). This is an old test based on polymerisation of amines present in the blood (fibrinogen, gamma-globulins) with aldehyde (glutaraldehyde) (Sandholm, 1974). The more the blood contains inflammatory proteins (fibrinogen, gamma-globulins), the faster the blood will coagulate in the sample tube. This test is mainly dedicated to chronic inflammation diagnosis or severe acute with hyper-fibrinogen. The test can be used for chronic traumatic reticulo-peritonitis, chronic pericarditis, chronic pneumonia, chronic peritonitis, chronic hepatitis, chronic pleurisy, chronic metritis, important abscess, chronic arthritis/polyarthritis, chronic nephritis/pyelonephritis, some mastitis (Metzner et al., 2007). The test must be reserved for cattle above 1 year-old. The test is not specific of a disease; the clinician must complete his clinical examination to find the area where inflammatory process occurs. In very severe acute process, the fibrinogen concentration can be enough to make the test positive. In most of the cases, the veterinarian has to count 7-10 days after the beginning of the disease, for the concentration of gamma-globulins to be enough to declare the test positive. This test is easy and cheap (~6€/test).

b. Estimated fibrinogen (*efibri*)
    This approximate method is based on the principle that the protein difference between serum and plasma, in a same blood sample, is mainly fibrinogen. It is a cheap method to evaluate fibrinogen, a positive acute marker of inflammation in cattle. A blood sampling has to be made in heparin and plain tube, centrifugation and lecture of protein amount using a refractometer. Beyond 7 g/L of difference between plasma and serum total protein, an acute inflammation may be suspected. The correlation between fibrinogen measured at the laboratory (measure of coagulation time of plasma + thrombin with coagulometer) and *efibri* is globally interesting to be used in the field (r=0.82) (Guyot et al., 2011).

c. Total protein in serum (TPS)
    Total protein in serum (TPS) can be assessed using a refractometer. The punctual measurement of TPS above 81 g/L (Radostits et al., 2007) may only lead to a suspicion of inflammation, as dehydration, haemolysis, hyper-uraemia or other factors can influence the result and interpretation. The TPS is linked to both acute and chronic inflammation.
    Total protein can also be measured in abdominal fluid (paracentesis) and discriminates transudate from exsudate. An exsudate will present a TP concentration above 30 g/L (Wittek et al., 2010).

According to a preliminary study (Guyot et al., 2011) to compare laboratory (haptoglobin, fibrinogen) and inflammation field tests (glutaraldehyde and efibri), there are good correlations between field and laboratory tests (p<0.05). The glutaraldehyde test has sensitivity (Se) of 100% and specificity (Sp) of 67% (Youden 0.67) for diagnosing inflammation, and *efibri* 72% (Se) and Sp (83%) (Y 0.55), compared to laboratory tests. If only one test has to be used, the glutaraldehyde is the best (and quickest) one. If *efibri* is added, the performances of diagnosis are increased.

Moreover, the presence of inflammatory cells can be detected, as an infectious agent is going to attack the host. These cells can be detected in numerous physiological fluids. An EDTA tube will be preferred for sampling to preserve the cells.

d. Presence of inflammatory cells (PMN)
   a. *Milk and colostrum*
       While infection in the mammary gland occurs, somatic cells increase in the udder to fight infection. This produces an inflammatory process. Assessment of inflammatory cells in colostrum or milk is an indicator of inflammation/infection in the mammary gland and allows the follow-up of infection or healing.
       The Californian Mastitis Test (CMT or Schalm's test) (Schalm and Noorlander, 1957) is the oldest test used to assess somatic cells in milk. The range of the test is 200,000 to 5,000,000 cells/ml. The principle is based on the formation of gel regarding the number of nucleus (cells), after addition of an anionic surfactant or detergent (reacting with nucleus). The more the aspect of milk looks like jelly, the more the content of somatic cells is important in the milk. Another method is to use an automatic cell counter (e.g. DCC DeLaval [~3,200€+3-4€/test], working with fluorescence or Ekomilk Scan [~1,500€+ 0.5€/test], working automatically with the principle of Schalm's test [formation of gel with nucleus present in milk]). These automatic counters work with ranges of measurement varying from 10,000 to 4,000,000 cells/ml (DCC Delaval) or 90,000 to 2,200,000 cells/ml (Ekomilk Scan).

   b. *Urine*
       As in the milk, the presence of somatic cells in urine signs inflammation and/or infection. Urinary strips are not reliable. The CMT and automatic cells counters can be used, depending of the number of cells in the urine. The Uriscreen® test is an interesting alternative. This test allows evidence of haematuria, bacteriuria (linked to bacteraemia in calves, according to Raboisson et al., 2010), pyuria and presence of somatic cells in urine, based on the detection of catalase activity. A layer of foam will appears after

addition of hydrogen peroxyde in positive urine samples. For bacteriuria, the Se and Sp of test are 86.6% and 88.8%, respectively. For bacteraemia in calves, the Se reaches 80% and Sp 92.8%.

c. *Cerebro-spinal fluid (CSF)*

The normal concentration of cells in the CSF is quite low ($<0.012.10^9$ cells/L). Somatic cells can increase in the CSF while infection/inflammation (Scott, 1995) and can be measured by automatic cell counter or CMT, if the cell concentration is enough.

d. *Articular puncture*

An increased concentration of somatic cells can be observed while arthritis, however, the usual tests (CMT, automatic cell counter) can be more difficult to interpret, as the articular fluid presents naturally a certain degree of viscosity. The cell concentration in a septic arthritis is above 10,000 cells/ml.

e. *Abdominal puncture*

The normal volume of abdominal liquid is 1 ml/kg body weight. In case of septic peritonitis, the number of somatic cells will also increase to exceed 10,000 cells/µl, as an exsudate (Wittek et al., 2010). Automatic cell counter and CMT can be used to determine the somatic cell concentration.

METABOLIC CONSEQUENCES

a. Blood

a. *Acid-base balance and ions*

These analysis can be undertaken in calves with diarrhoea or cows with ileus. The pH, Base Excess (BE), bicarbonate (HCO_3^-), Na^+, Cl^-, K^+ are of particular interest for diagnosis, prognosis and therapeutics. Indeed, metabolic acidosis or alkalosis must be treated differently and can be sometimes very difficult to differentiate clinically. In the same way, ionic disorders are essential to diagnose for discrimination of gastro-intestinal troubles in calves and adult cattle. For example, calf diarrhoea is often associated with metabolic acidosis, hyperkalieamia and hypoglycaemia. But metabolic alkalosis can be met in the same condition, and cannot be clinically distinguished. An ileus in adult cattle, such as a left displacement of abomasum (LDA), usually leads to metabolic alkalosis, hypokaliaemia and hypochloremia, with or without paradoxal aciduria, but in some conditions, a metabolic acidosis can be observed as well. Many devices are now available for field utilization, at acceptable prices. In the market, there are different tools varying from 7-10,000€, and 10-20€/test

(with pH, BE, bicarbonate, ions, glucose, lactate, etc.). The results are available within 5 minutes, at cow or calf-side, in the barn.

These devices also provide haematocrit and haemoglobin concentration. This can be helpful to control anaemia, haemolysis, or haemorrhages.

b. *Glucose*

Glucose reflects energy metabolism but is very variable. Its interest lies in neonatalogy, in ketosic cows or after infusions. Glucose may help to discriminate the different types of ketosis (**Table 1**). It is easily and quickly (5 seconds) measurable on the field with very cheap portable glucometers (~75€ + 1€/test). The Se and Sp are good but the Se decreases with glucose concentration under 40 mg/dl. The analysis must be performed immediately after blood sampling (complete blood). The normal range for a healthy cow is 54-110 md/dl.

c. *Beta-Hydroxy-Butyrate (BHB)*

BHB is one of the three ketone bodies in cattle. The interest of its assessment is to evaluate the different types of ketosis (**Table 1**), negative energy balance or other metabolic diseases (fatty liver syndrome, fasting, metritis, LDA). The same device as used for glucose can be taken, with different reactive strips. The results appears in 10 seconds for an approximate cost of 3-5€/test. The range of measurement is 0-8 mmol/L. This also requires an immediate analysis on complete blood. It is indicated to take blood samples 4-5h after cow's meal. In general, the cut-off chosen for subclinical ketosis is >1.2 mmol/L. BHB can also be measured in milk, as well as aceto-acetate, using milk strips, but with lower Se/Sp compared to blood-BHB (Geishauser et al., 1998). Pre-partum negative energy balance is highlighted with BHB >0.6 mmol/L in cows at the end of pregnancy.

Table 1: indicative levels of glucose & BHB in cows with type I and II ketosis.

	Type I ketosis	Type II ketosis
Glucose	↓ or Normal	Normal or ↑
BHB	↑↑ to ↑↑↑↑	↑ to ↑↑

d. *L-Lactate*

Lactate has two isomers: D and L lactate. L-Lactate is a product of sugar metabolism in mammals and metabolized by liver. This isomer is a reflection of anaerobic metabolism. D-Lactate is produced by bacteria and has a slow elimination. On the field, only the L-Lactate can be assayed, but not D-Lactate.

The assay on the field is made by a small "side-cow" spectrophotometer (100-400€, ~3€/test, result in 1 minute), with a range from 0.8-21.7 mmol/L. There is a small overestimation (0.5 mmol/L) above 3 mmol/L. The animal must be quiet before sampling. The analysis, on complete blood, must be performed immediately after blood sampling. L-Lactate in cows is considered normal if less than 1.5 mmol/L, at rest.

L-Lactate can be used as a prognosis factor for infectious broncho-pneumonia (BP) in young cattle (up to 13 months), with poor prognosis for calves with type IV BP and L-Lactate ≥ 4 mmol/L (Se 95%, Sp 80%) (Coghe et al., 2000). Other studies show similar results with 44 times more risks to die in calves with L-Lactate > 3.7 mmol/L, compared to calves with L-Lactate around 1.3 mmol/L (Buczinski et al., 2014). Regarding calf diarrhoea, L-Lactate measurement is unhelpful, as most of the time, it is D-Lactate that is produced and accumulated (Ewaschuk et al., 2004). Finally, L-Lactate can be assessed in colic (volvulus, gasto-intestinal ileus, intussusception, etc.) as a prognosis factor. During pre-surgery assay, a L-Lactate > 6 mmol/L may lead to the prevision of a negative outcome (Se 29%, Sp 98%) and L-Lactate < 2 mmol/L predicts a positive outcome (in 93% of cases) (Boulay et al., 2013). L-Lactate post-surgery seems not to be a better indicator.

b. Abdominal fluid

a. *Glucose*

The glucose concentration in plasma is similar to abdominal fluid and situated between 42 and 133 mg/dl. In case of septic peritonitis, bacteria will consume glucose and will decrease glucose concentration in abdominal fluid. The cut-off is set at 31 mg/dl or if plasmatic glucose concentration is higher of 20 mg/dl while compared to abdominal fluid (Wittek et al., 2010).

b. *L-Lactate*

The L-Lactate concentration in plasma is also similar to abdominal fluid and situated between 0.19 and 1.31 mmol/L. When intestinal or abomasal ischaemia occurs, there is a synthesis of L-Lactate due to anaerobic conditions and the L-Lactate concentration in abdominal fluid will increase dramatically (Wittek et al., 2010). Bacteria will also produce D-Lactate that cannot be measured.

c. Feces

a. *pH*

pH of feces has an interest in calf diarrhoea to discriminate hypersecretion diarrhoea from malabsorption diarrhoea. The pH is measured in a solution of 15 grams of feces mixed with 85 ml water. A pH above 7 will conclude to a

hypersecretion of HCO_3^-, while a pH <6 reveals the presence of acid organic molecules, and a non-effective absorption, as well as neofermentation in the large intestine.

IMMUNITY CONSEQUENCES

a. Antibodies assays

   a. *Transfer of colostral immunity (TCI)*

   Failure of TCI will get negative outcome on calves' health. In dairy cattle, a decrease of average daily gain and subsequent milk production (1st lactation), as well as an increase of mortality and culling (1st lactation), is observed while failure of TCI (Robinson et al., 1988; DeNise et al., 1989). In beef cattle, failure of TCI will lead to an increased morbidity (especially respiratory troubles), calf mortality (pre-weaning), and decreased average daily gain, compared to cattle with correct TCI (Wittum and Perino, 1995). It should be noted that increased intakes of IgG in calves from a herd with a correct status of TCI will not result in reduction of mortality and morbidity.

   There are different ways to asses correct or failure of TCI. Both ways are focused on blood sampling in healthy calves at an age between 2 and 6 days of life. The gold-standard is the Radial-Immuno-Diffusion (RID) method but this is not performable on the field.

   The indirect method consists in measuring total protein in serum, using a refractometer. It is not really a calf-side test and it needs material. Failure of TCI is set if TPS are below 56 g/L. Calves must not be sick or dehydrated. TPS reflects approximately the IgG concentration but the proportion may vary according to animals.

   The direct method, on the field, is a semi-quantitative test based on the coagulation of gamma-globulins with glutaraldehyde. It needs no further material (cost ~6-7€/test). The test, developed by the Ambulatory Clinic of the University of Liège (Calf-IgG-Test, distributed by NBVC) has a Se of 92% and Sp of 71% (compared to the gold-standard). The performance of the test is calculated to limit the falsely negative samples. A coagulation time within one minute assumes an adequate transfer of immunity in the young healthy calf (blood IgG concentration > 10.1 g/L).

   b. *Colostrum quality*

   In the same way of the assessment of TCI, colostrum quality can be measured in a direct or indirect method.

   The indirect method requires a colostrumeter (measuring colostrum density). Assuming that about 50-60% of total proteins in the colostrum are IgG, a good

colostrum must contain at least 75 g/L of total protein. A specific refractometer can also be used on that purpose.

The direct method, on the field, is also a semi-quantitative test based on the coagulation of gamma-globulins with glutaraldehyde. Fresh colostrum is mixed with a solution of glutaraldehyde and coagulation time is noticed. A quick coagulation time indicates a good quality colostrum (> 50 g/L IgG). The test is actually under development by the Ambulatory Clinic of the University of Liège, with actually similar performances (Se/Sp) compared to Calf-IgG-Test.

THE INFECTIOUS AGENT

In the frame of this congress, only quick tests that can be performed within 15 minutes, cow or calf-side, are presented. The laboratory remains the gold-standard, with the best Se-Sp and accuracy values. There will not be discussion about office milk bacteriology, parasitology, and blood smears.

a. Antibodies immunoassays
   a. *Feces*
      Lateral immunochromatography is mainly used to determine infectious agent in calf diarrhoea. Numerous tests are available, with prices varying between 10-30€ and results in 15 minutes. The main infectious agents that can be found with these tests are *Escherichia coli* (K99, CS31A), *Rotavirus*, *Coronavirus*, *Cryptosporidium*, *Clostridium perfringens*, *Giardia*. The Se and Sp may vary according to manufacturers and are located between 64-100%, and 92-100%, respectively. The practitioner has to take into account the age of the calf and the treatments that may have been administered to the calf, before interpretation of the results.

b. Evidence of the biological agent
   a. *Skin scraping*
      This easy method can bring out skin parasites, just by scraping the skin up to blood dew, and observing on a blade with one drop of chlorolactophenol under a microscope (small magnificience, X10). *Psoroptes ovis*, *Sarcoptes scabiei*, *Chorioptes bovis* but also lices (*Haematopinus*, *Linognathus*, *Damalinia*) can be identified by this method.

CONCLUSIONS

The diagnosis of a disease begins with a good history and a correct clinical examination. The ancillary exam confirms the clinical suspicion and it is not the result of the ancillary exam

who leads to the clinical suspicion. Even though cattle practice is economic medicine, veterinary practitioners must promote the use of ancillary examinations as prognosis factor first, but also as a diagnostic and pedagogic tool for the farmer. These examinations allow the practitioner to communicate and show they are investigating, with new technologies, the individual or herd troubles. Moreover, some infectious or metabolic diseases can co-exist or be subclinical. In these cases, ancillary exams are essential to go further with diagnosis, prognosis and communication in the farms.

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