Diagnosis of neonatal calf diarrhoea

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SUMMARY

Neonatal calf diarrhoea (NCD) is the most important disease of neonatal calves and results in the greatest economic losses due to disease in this age group in both dairy and beef calves. NCD outbreak associated costs include treatment time, possible impact on subsequent calf growth performances and potential death. NCD results from a combination of an adverse environment, poor host immunity and challenge from infectious agents. While an etiological diagnosis in NCD cases may not be relevant for the treatment of the individual, such diagnosis helps determining which adapted measures can be taken on herd level especially if zoonotic pathogens are involved. The clinical diagnosis should be based on the clinical picture including the age of the affected animals and associated clinical signs collated through a thorough physical examination together with epidemiological data including the season and herd history. In most situations, the clinical diagnosis requires confirmation by on-farm or laboratory tests.

Keywords: Calf, diarrhoea, diagnosis, herd, epidemiology, clinical signs, confirmation, laboratory.

RÉSUMÉ

Diagnostic des diarrhées néonatales du veau

Les diarrhées néonatales du veau constituent la maladie la plus importante des veaux nouveau-nés, entraînant les pertes économiques les plus élevées dans cette classe d’âge, tant chez les veaux laitiers que chez les veaux allaitants. Les coûts associés aux diarrhées néonatales du veau comprennent le temps dévolu au traitement, l’impact possible sur les performances de croissance du veau et la mortalité. Les diarrhées néonatales du veau résultent de la combinaison de facteurs environnementaux défavorables, d’une immunité faible de l’hôte et d’agents infectieux. Alors qu’un diagnostic étiologique des cas de diarrhées néonatales se révèle peu important sur le plan du traitement individuel, en revanche il aide à déterminer quelles sont les mesures adaptées à prendre au niveau du troupeau, surtout si des agents zoonotiques sont impliqués. Le diagnostic clinique devrait être fondé sur les données cliniques, comprenant l’âge des animaux atteints et les symptômes associés relevés grâce à un examen clinique consciencieux, ainsi que des données épidémiologiques incluant la saison, et l’historique du troupeau. Dans la plupart des situations, le diagnostic clinique requiert une confirmation par des tests réalisables dans la ferme ou avec l’aide du laboratoire.

Mots clés : Veau, diarrhée, diagnostic, troupeau, épidémiologie, signes cliniques, confirmation, laboratoire.

Introduction

Diarrhoea is characterized by the more frequent production of faeces that are wet and soft (less than 10% dry content). This clinical sign is easy to diagnose but non specific. Effectively, it is encountered in a number of diseases of the young animal, which are due to several agents; these agents can act concomitantly to produce the disease in the calf.

Often, this group of diseases is named “enteritis”, although classical inflammation of the mucosa may not be present. For instance, when diarrhoea is associated with enterotoxigenic strains of E. coli, there is mainly a large net increase of secretion due to enterotoxins, with minor lesions of the gut mucosa.

Diarrhoea is the most important disease of neonatal calves and results in the greatest economic loss due to disease in this age group in both dairy and beef calves. Outbreaks of neonatal calf diarrhoea (NCD) can be indeed costly, especially for beef producers, as treatment of affected calves is time consuming, and the death of a calf represents the loss of profit from that cow–calf unit for the year. Additionally for survivors there may be impacts on subsequent calf growth rate and weaning weight, loss of genetic potential and a decreased capacity to improve and maintain the herd.

NCD is generally observed together with other clinical signs, among them essentially dehydration with acid-base imbalance. Dysentery or even abdominal pain (colic) can be seen. All this depends on the cause of diarrhoea, on the pathogenesis (enteritis, malabsorption…), on the location and severity of corresponding lesions…

At the individual level, the precise diagnosis is not always indispensable, because it does not affect the course of actions to take (generally urgent rehydration followed by a number of hygienic measures and supportive treatment).

But at the herd level, diagnosis is important, and it has consequences in question of applied treatment or vaccination. Aetiological diagnosis of calf scours has to be made as rapidly as possible. Effectively, as infectious agents are generally implicated, it is of importance to check what agent(s) is (are) circulating in the herd and is/are mainly determining the observed outbreak. An adapted treatment may be proposed after diagnosis and above all the diagnosis may help defining an adapted prophylaxis based on vaccination of dams or calves as well as modification of management of the herd in the areas of feeding, control of parasitism, etc.

Finally it is also of importance to check whether the outbreak is due to a zoonotic pathogen such as Salmonella, Campylobacter…

Therefore, in order to adapt therapeutics and prophylactic advices, it is of importance to search for the precise aetiology of the observed digestive disorders.

**Epidemiology**

Neonatal calf diarrhoea (NCD) occurs mainly in calves less than six weeks of age, although calves up to four months of age may be affected. It can affect beef as well as dairy calves. It occurs mainly also during the winter period and when animals are within stables [5]. Lots of animals can be affected: for instance, in a French study on beef calves, the mean incidence rate for diarrhoea during the neonatal period was 14.6%, with herds having an incidence over 50% [2].

During the winter season, the number of diarrhoeal calves increases often together with the severity of the disease. This is linked to the fact that the infection pressure for neonates tends to increase progressively. A number of key points can explain this phenomenon. First, all major enteric pathogens are commonly carried by asymptomatic adult cows and shedding of many pathogens species tends to increase around calving. Second, healthy calves are often asymptomatically or subclinically infected with enteric pathogens and amplify environmental contamination. Finally, all major enteric pathogens can survive in the environment and water sources for weeks to months (and in most cases over a year) in cool, damp conditions.

Many other animals, including feral animals and domestic pets, can also contribute to the contamination of calves by pathogens like *Cryptosporidium, Salmonella* or rotavirus.

Because of its essential infectious aetiology, NCD incidence increases in herds where infection pressure increases and in herds where calves have an inadequate immunity: this can be due to a failure of transfer of colostral immunity or to stressing factors like a cold weather [1, 16].

**Aetiology**

NCD is a multifactorial disease and results from a combination of an adverse environment, poor host immunity and challenge from infectious agents [1, 16]. Although a very serious impairment in any one of these factors can lead to disease, NCD outbreaks usually reflect a problem in all three areas. Consequently it is important to address the problem at a herd level by assessing risk factors and implementing preventive management.

**ADVERSE ENVIRONMENT**

Adverse conditions in the environment of the neonate can contribute to the emergence of diarrheal outbreaks. For instance, a cold winter with air drafts falling on calves, a wet litter, a litter made of granite fines are risk factors associated with NCD [15]. Indeed, environment of neonates is a key factor in the emergence of diarrhoea: housing with abundant straw bedding, nature of the pen walls, air volume per animal [12]… Also, the absence of cohabitation with animals of other age classes is important to control.

One must not forget that diarrhoea may have also a nutritional origin. This can be linked to the distribution of raw milk at unsuitable temperatures, or of too large quantities (over 3 L per meal). This can be linked also to the use of milk replacers of inferior quality, or badly prepared (with lumps…). All this can lead to the appearance of diarrhoea in the calves, with often great quantities of soft faeces. But, initially, there is no or minimal systemic effect… until there is superinfection by a pathogen.

**POOR HOST IMMUNITY**

A poor immunity in neonatal calves is essentially linked to a failure of passive transfer (FPT) of colostral antibodies. This may be due to an insufficient or delayed ingestion of colostrum by the neonate or to a poor quality of the colostrum [11, 13-14]. Adequate ingestion of the colostrum by the neonate can be easily controlled by a careful farmer. Colostrum quality can be checked with a colostrometer; this quality is conditioned by the parity and the sanitary status of the dam, linked to the control of parasitism, the nutrition, the season [9]…

**CHALLENGE FROM INFECTIOUS AGENTS**

Several pathogenic agents can, alone or, most often, in combination, determine diarrhoea in the young calf. These agents can also act successively in the same calf: this can explain discrepant laboratory results of successive analyses for a single calf or between calves in the same herd. Enteropathogenic agents are usually present in the environment of the calves or are carried and shed by the dam or other animals from the herd: sick or convalescent animals, asymptomatic carriers [5, 18]… During calving season, the concentration of pathogens in the environment increases progressively, with an amplification by the first calves of the season. This increases infectious pressure, and, associated with favourable conditions, this leads to a degradation of the health of calves and to the emergence of real neonatal diarrhoea epizootics.

The main agents responsible for diarrhoea in the calf [16] (Table I) are bacteria (mainly *Escherichia coli* strains, Table II), parasites (essentially *Cryptosporidia*) and finally viruses (among them particularly rotaviruses and to a less extent coronaviruses).

Enteropathogenic agents are very frequently isolated from faeces of diarrhoeic calves that were not preliminarily treated with antibiotics, *E. coli* being the commonest. *E. coli* is isolated alone in half of the cases but a sequential action of several agents can not be excluded. In other cases, *E. coli* is isolated along with other agents: most often associated pathogens are by decreasing importance *cryptosporidia*, *rotavirus*, coronaviruses, and toroviruses [5, 6, 10].

Finally an essential point must be underlined: whatever the responsible pathogen(s), diarrhoea is frequently associated in
the young calf (from the birth to 3-4 weeks of age) to a toxaemia and/or a septicaemia that complicate the disease and worsen the prognosis.

Clinical diagnosis

Practical diagnosis will first rest on the thorough clinical examination of affected calves, together with a precise anamnesis.

The aspect of diarrhoea can help orientating the diagnosis, which will be based on associated signs as well as the age of the affected animals (Table III). Effectively, each neonatal disease characterised by diarrhoea in the calf has its peak incidence at a specific age (Figure 1). Colibacillosis is generally

**TABLE I: Main pathogenic agents responsible for diarrhoea in the calf (non exhaustive list).**

<table>
<thead>
<tr>
<th>Pathotype</th>
<th>ETEC (entero-toxigenic E. coli)</th>
<th>VTEC (vero-toxigenic E. coli)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>EPEC (entero-pathogenic E. coli)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age of affected calves</td>
<td>&lt; 5 days</td>
<td>1 to 3 weeks</td>
<td>1 to 3 weeks</td>
</tr>
<tr>
<td>Adhesins</td>
<td>K99 (F5), F41, FY (F5), CS31A</td>
<td>Intimin (Eae)</td>
<td>BFP (“bundle forming pili”)</td>
</tr>
<tr>
<td>Toxins</td>
<td>STa (ST1)</td>
<td>VT1 and VT2 (Stx1, Stx2)</td>
<td>-</td>
</tr>
</tbody>
</table>

<sup>a</sup> mainly EHEC: enterohaemorrhagic E. coli; can be named STEC (shigatoxic E. coli). Responsible for HUS (haemolytic and uremic syndrome) in the child under 5 years, mainly serotype O157:H7.

<sup>b</sup> mainly AEEC: adhering-effacing E. coli.

**TABLE II: Main Escherichia coli pathotypes responsible for calf diarrhoea and associated virulence factors [17, 23].**

<table>
<thead>
<tr>
<th>Mean age of affected calves</th>
<th>Clinical signs</th>
<th>Probable aetiologcal diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-3 days</td>
<td>Very liquid diarrhoea, yellow</td>
<td>Colibacillosis (ETEC = F5 E. coli)</td>
</tr>
<tr>
<td></td>
<td>Rapid and important dehydration (eyes sinkness, diminished skin elasticity)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Weakness, cold extremities</td>
<td></td>
</tr>
<tr>
<td>4-11 days</td>
<td>Mucoi diarrhoea</td>
<td>Rotavirosis, coronavirosis, cryptosporidosis</td>
</tr>
<tr>
<td></td>
<td>Hyperthermia</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anorexia, abdominal pain</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Progressive dehydration</td>
<td></td>
</tr>
<tr>
<td>&gt; 11 days</td>
<td>Very liquid diarrhoea with blood traces</td>
<td>Salmonellosis</td>
</tr>
<tr>
<td></td>
<td>Severe hyperthermia (&gt; 41°C)</td>
<td></td>
</tr>
<tr>
<td>&gt; 18 days</td>
<td>Black diarrhoea, +/- blood and colic</td>
<td>Coccidiosis due to <em>Eimeria zuernii</em></td>
</tr>
<tr>
<td></td>
<td>Mucoi diarrhoea, hyperthermia</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ptyalism, anorexia, epiphora</td>
<td>Bovine viral diarrhoea</td>
</tr>
</tbody>
</table>

**TABLE III: Helpful elements for the differential diagnosis of NCD.**
encountered in very young calves (under 5 days) while cocci
diosis (eimeriosis) will affect preferentially calves over 3
weeks.

Because of the pathogenic course of diarrhoea, the affected
animal will generally experience dehydration together with a
more or less severe acidosis linked to either faecal losses of
bicarbonate or L-lactate production following dehydration or
D-lactate production by Lactobacilli. The age of the calf
plays a great role in the susceptibility of the calf to the diar-
rhoea. Dehydration and acidosis (especially D-lactic acidosis
occurring in the “diarrhea without dehydration syndrome” in
10-day-old calves) have systemic consequences and lead to
depression, recumbency, or even cardiac or renal failure…
Hypothermia or cachexy may also be observed in affected
calves.

Together with the age of the affected calf, the observed
systemic effects and their severity can orientate the practitio-
nert towards the suspected responsible enteropathogen. For
instance, a marked hyperthermia (> 40.5-41°C) may lead to
a suspicion of colibacillosis, salmonellosis or coronavirus,
as well as a high mortality of affected calves. By contrast, a
low mortality is rather evocative of rotavirosis. We may note
also that the absence or presence of hyperthermia in the
affected calf determines the choice for the adapted treatment,
taking into account the responsible and prudent use of anti-
biotics [4].

When NCD is accompanied by mortality, the practitioner can
obtain interesting hints by realising a necropsic examination:
observed lesions and samples can be very helpful to determine
the aetiologic agent. Necropsy will also aid evidencing the
possible implication of the suspected pathogen in the diarrheal
episode: one must keep in mind that the presence of an ente-
ropathogen in an affected calf is not enough to prove its role in
the pathogenic process. Indeed, asymptomatic carriers and shed-
ders exist, and the evidence of associated lesions is necessary
to demonstrate the implication of a suspected pathogen.

Paraclinical diagnosis

The clinical examination in the farm leads to a suspicion
towards the implicated pathogen, together with a number of
risk factors contributing to the outbreak. The practitioner
needs afterwards to confirm his suspicion with the help of
laboratory tests (Table IV). Among them, several can be carried
out by the practitioner in the field or in his clinic (Table V).

Moreover, when faced to an outbreak of NCD in a herd
one must verify FPT by measuring gamma-globulinemia in
calves [13, 14, 20, 22]. A number of dedicated commercial
tests that can be carried out in the field is today available
(Table V, first lines). Once the FPT is diagnosed, the practi-
tioner will help improving the passive transfer of immuno-
globulins in calves [7, 8].

<table>
<thead>
<tr>
<th>Agent</th>
<th>Sample</th>
<th>Carried out method - observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>Faeces, intestinal content</td>
<td>Elisa « BVT »</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Culture then slide agglutination (F5, F41, F17, CS31A)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Antibiotic resistance profile.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Search for genes specific for septicaemic E. coli [19]</td>
</tr>
<tr>
<td>Salmonella</td>
<td>Faeces; on dead animal: ileo-colon, mesenteric lymphatic nodes, spleen,</td>
<td>Culture, then antibiotic resistance profiling and serotyping</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Liver (aborted foetus)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Serology: no diagnostic interest</td>
</tr>
<tr>
<td>Clostridium</td>
<td>Faeces, intestinal content (hermetically sealed flacon), 4°C, brief delay for analysis</td>
<td>Culture and enumeration</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Possible search for genes coding for toxins (PCR)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Do not forget C. difficile ! [21]</td>
</tr>
<tr>
<td>Rotavirus</td>
<td>Faeces; on dead animal: ileon</td>
<td>Elisa « BVT »</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Immuno-enzymatic search, latex test</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ph: only group A rotaviruses</td>
</tr>
<tr>
<td>Coronavirus</td>
<td>Faeces; on dead animal: ileo-colon</td>
<td>Elisa « BVT »</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Immuno-enzymatic search, agar immuno-diffusion</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sensitivity 10⁶ PFU/g</td>
</tr>
<tr>
<td>Adénovirus</td>
<td>Faeces</td>
<td>Human ELISA kits useful [3]</td>
</tr>
<tr>
<td>Caliciviruses</td>
<td>Faeces</td>
<td>RT-PCR for identification [24] based on group primers</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Human ELISA kit useful because of the antigenic community.</td>
</tr>
<tr>
<td>Torovirus</td>
<td>Faeces</td>
<td>ELISA and RT-PCR have been described [10]. Possible development of a multiplex RT PCR for cattle:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>calicivirus and adenovirus (+ astrovirus)</td>
</tr>
<tr>
<td>Pestivirus BVD/MD</td>
<td>Faeces: NO! Naso-pharyngeal lavage, blood (PI only); on dead animal: ileo-colon, lymphatic nodes, spleen, abomasum, rectum, lung (aborted foetus)</td>
<td>Immuno-enzymatic test in 24h (Antigen), cellular culture (gold standard: 8-21d), immunofluorescence on organs (1-3d delay)</td>
</tr>
<tr>
<td>Cryptosporidium</td>
<td>Faeces</td>
<td>Microscopic examination (Ziehl Neelsen, Heine…)</td>
</tr>
<tr>
<td>Eimeria (bovis, zuernii)</td>
<td>Faeces</td>
<td>Microscopic examination</td>
</tr>
</tbody>
</table>

Table IV: Samples to be done according to the suspected enteropathogen in face of calf diarrhoea.
Tigettes tétravalentes » Differential diagnosis of NCD for 4 agents (virus, bacteria, Bio K 156 Faeces Immunochromatography 15 minutes or a precise necropsy, the diagnosis can be confirmed. or in the clinic, or even with the help of laboratory analyses with the help of rapid tests that can be carried out in the farm to hypothesize what is the probable diagnosis. Afterwards, the aspect of the faeces and the associated clinical signs help the affected animals: the age of affected calves, together with is also useful when the suspected agent is of public health treatment but also and above all the preventive measures. It herds where the infection pressure increases, to adapt the surgical diagnosis is difficult to carry out. It is indispensable in


Conclusion

In conclusion, NCD remains the most important disease in calves under 4 weeks, responsible for huge economical losses. Several enteropathogens can act successively or together to determine NCD in herds where risk factors are present, which impair the calf’s immunity and especially contribute to FPT.

Although NCD is quite easy to identify, a precise aetiological diagnosis is difficult to carry out. It is indispensable in herds where the infection pressure increases, to adapt the treatment but also and above all the preventive measures. It is also useful when the suspected agent is of public health concern.

In order to carry out a precise diagnosis, the practitioner needs first to carry out thoroughly a clinical examination of the affected animals: the age of affected calves, together with the aspect of the faeces and the associated clinical signs help to hypothesize what is the probable diagnosis. Afterwards, with the help of rapid tests that can be carried out in the farm or in the clinic, or even with the help of laboratory analyses or a precise necropsy, the diagnosis can be confirmed.

References


TABLE V: Available commercial tests helpful in the diagnosis of NCD.

<table>
<thead>
<tr>
<th>Test (supplier)</th>
<th>Sample</th>
<th>Method / result</th>
<th>Delay for obtention of results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine® IgG (Midland)</td>
<td>Colostrum</td>
<td>Immunochromatography Evidence of a quantitative failure of IgG secretion by the udder</td>
<td>20 minutes</td>
</tr>
<tr>
<td>Calf® IgG (Midland)</td>
<td>Total blood</td>
<td>Evidence of a quantitative failure of IgG in the calf’s blood</td>
<td>20 minutes</td>
</tr>
<tr>
<td>Speed® Giardia (Bio Véto test)</td>
<td>Faeces</td>
<td>Immunochromatography Evidence of Giardia duodenalis oocysts in a weight loss syndrome with intermittent diarrhoea in the young calf</td>
<td>5 minutes</td>
</tr>
<tr>
<td>Speed® V diar 4 and Speed® V diar 5 (Bio Véto test)</td>
<td>Faeces</td>
<td>Immunochromatography Differential diagnosis of NCD for 4-5 agents (rota/coronavirus, C. parvum, E. coli K99, E. coli CS31A)</td>
<td>15 minutes</td>
</tr>
<tr>
<td>Bio K 156 « Tigettes tétravalentes » (Bio-X Diagnostics)</td>
<td>Faeces</td>
<td>Immunochromatography Differential diagnosis of NCD for 4 agents (virus, bacteria, parasites)</td>
<td>15 minutes</td>
</tr>
<tr>
<td>Bio K 195, Bio K 170, Bio K 176 (Bio-X Diagnostics)</td>
<td>Faeces</td>
<td>Diagnosis of, respectively, Clostridium perfringens and its α and ε toxins</td>
<td>15 minutes</td>
</tr>
</tbody>
</table>


