PROCEEDINGS

BARCELONA / SPAIN / 8-9 JUNE 2009

NEONATAL HEALTH IN CALVES

Comprehensive solutions for complex enteric disorders

Intervet
Schering-Plough Animal Health

Université de Liège

8th of June - Afternoon session

Chairman: Prof E. Thiry

Pathogenesis

Opening lecture: Neonatal diarrhea in calves - a multifactorial disease
Wolgang Klee

Bovine coronavirus, torovirus and rotavirus infections
Nicola Decaro

Bovine enteric caliciviruses- are they relevant?
Janice Bridger

Bacteria associated with neonatal diarrhoea- a new perspective?
David Smith

Parasites involved in neonatal diarrhoea
Arwid Daugschies

Pathomorphology of the intestinal barrier in bovine neonatal diarrhea
Elisabeth Liebler Tenorio

Experimental infection of antibody positive calves with bovine rota- and coronaviruses
Birgit Makoschey

Clinics and diagnosis

Neonatal diarrhea: Clinical diagnosis and sampling
Yves Millemann

Laboratory diagnosis of neonatal calf diarrhea
David Snodgrass

On-site diagnosis of cryptosporidiosis and giardiasis in calves
Edwin Clarebout

9th of June - Morning session

Chairman: Prof E. Thiry

Epidemiology

Risk factors for neonatal diarrhea in beef cattle
Fatah Bendali

Pathogens in faeces of calves aged from 1-21 days in Dutch dairy herds; Prevalence and risk factors
Jeroen van de Ven

Rotavirus and concurrent infections with other enteropathogens in neonatal diarrheic dairy calves in Spain
Angel Garcia

Epidemiology of neonatal diarrhea in dairy and beef calves in Czech Republic
Josef Illek

Impact of frequent calf diseases on subsequent productivity
Martin Kaske

Prevention and Treatment

Neonatal calf health management
Keith Cutler

Colostrum management
Juan Vicente González

Prevention and treatment of cryptosporidiosis in calves- special emphasis on the use of Halofuginone Lactate in Portugal
Luis Madeira Carvalho

Enhancement of passive protection against bovine enteric coronavirus using Rotavec Corona
Collin Crouch

Index

Program 2

Neonatal diarrhoea in calves - a multifactorial disease
Wolfgang Klee 6

Bovine coronavirus, torovirus and rotavirus infections
Nicola Decaro 7

Bovine enteric caliciviruses - are they relevant?
J. C. Bridger 8

Bacteria associated with neonatal diarrhoea - a new perspective?
David GE Smith 9

Parasites involved in neonatal diarrhoea
Arwid Daugschies 10

Pathomorphology of the intestinal barrier in bovine neonatal diarrhea
Elisabeth M. Liebler-Tenorio 11

Experimental infection of antibody positive calves with bovine rota- and coronaviruses
Birgit Makoschey, Marina Timmermans, Petra Tonen and Ulrike Schmidt 12

Neonatal diarrhea: clinical diagnosis and sampling
Yves Millemann 13

Laboratory diagnosis of neonatal calf diarrhoea
David Snodgrass 14

On-site diagnosis of cryptosporidiosis and giardiasis in calves
Thomas Geurden, Jozef Vercruysse, Edwin Claerebout 15

Risk factors for neonatal diarrhea in beef calves
Fatah Bendali 17

Pathogens in faeces of calves aged from 1-21 days in Dutch dairy herds; Prevalence and risk factors
Menno Holzhauer, Chris JM Bartels, Theo Lam, Jeroen van de Ven and Ton de Gee 19

Rotavirus and concurrent infections with othe enteropathogens in neonatal diarrheic calves in Spain
Angel Garcia 20

Epidemiology of neonatal diarrhea in dairy and beef calves in the Czech Republic
Josef Illek 21

Impact of frequent calf diseases on subsequent productivity
Martin Kaske 22

Neonatal calf health management
Keith Cutler 23

Colostrum management
Juan V. González-Martín 24

Prevention and treatment of cryptosporidiosis in calves - special emphasis on the use of halofuginone lactate in Portugal
L.M. Madeira de Carvalho, S. Martins, S. Sousa, J. Bacelar, J. Cannas da Silva 25

Enhancement of passive protection against bovine enteric coronavirus using Rotavec Corona ™
Colin F Crouch 26

Neonatal Diarrhoea in Calves - a Multifactorial Disease

Wolfgang Klee

Clinic for Ruminants with Ambulatory Clinic and Herd Health Management
University of Munich

The infectious agents associated with neonatal calf diarrhea (NCD) are dealt with elsewhere in this symposium. While they are necessary "causes" of an outbreak of NCD, their presence is often not sufficient. Other factors determine both incidence and course of NCD, and will be the focus of this presentation. Without any doubt, the most important "non-infectious" factor is the human factor with its many facets. Many of them are interrelated with general aspects of infection.

Table 1: Quantitative factors that influence incidence of NCD

As factor increases	Incidence of NCD
Proportion of assisted calvings	↑
Proportion of weak calves	↑
Time to first colostrum feeding after birth	↑
Number of colostrum meals in first 24 h	↓
Volume of colostrum consumed in first 24 h	↓
Duration of colostrum feeding	↓
Daily increments in volume of meals	↑
Time to access to hay	↑
Intervals between use of hutches	↓
Number of calves per hutch	↑

Table 2: Qualitative factors that influence incidence of NCD

Factor	Incidence of NCD
Calving in calving pen	↑
Calves cared for by owner's wife	↓
Milk temperature checked with thermometer	↓

Increases in the following factors were associated with an increase in the case fatality: Number of calves per year, rate of deterioration of appetite, rate of deterioration of general condition, incidence of watery diarrhea, incidence of blood in feces, incidence of foul smell of feces, duration of diarrhea, number of calves per bucket, number of complicating diseases. Veterinarians have to concentrate on those factors that management can change.

Bovine coronavirus, torovirus and rotavirus infections

Nicola Decaro

Department of Veterinary Public Health, Faculty of Veterinary Medicine of Bari, Italy

Coronaviruses and rotaviruses represent the most common viral agents of neonatal diarrhoea in calves. Bovine coronavirus (BCoV) is a group-2 coronavirus (CoV) associated with different clinical forms of disease in cattle, including enteritis in newborn calves, "winter dysentery" in adult cows, and respiratory signs in calves and cows. BCoV can induce simultaneously enteric and respiratory disease in the same group of animals, regardless of the age. BCoV is believed to be able to cross the species barrier easily, since bovine-like CoVs have been detected in other mammalian species. A bovine origin has been hypothesized for the human coronavirus (HCoV) OC43, the porcine haemagglutinating encephalomyelitis virus (PHEV), the canine respiratory coronavirus (CRCoV), and for other ruminant CoVs.

Group A rotaviruses (GARVs) are segmented double-stranded RNA viruses with a non-enveloped virion. GARVs are important enteric pathogens in humans and animals. Since the immunity raised to GARV appears to be mainly serotype-specific, vaccines targeting the most common VP7 (G) and VP4 (P) antigens have been developed and licensed for prevention of GARV-associated diarrhoea in children. GARV infection in young calves is associated with severe enteritis and bovine GARV vaccines are also available. Direct inter-species transmission and adaptation of animal GARVs to humans via genetic reassortment contribute to diversification of human GARVs. Epidemiological studies worldwide have revealed dynamics interactions between human and animal GARVs, and bovine-like GARVs, G6P[9], G6P[14], G8P[14], G10P[14] have been reported sporadically in several countries. By converse, the bovine-like G10P[11] strain is common in children in India.

Toroviruses are emerging viruses that have been associated to acute enteritis in neonatal calves. The prototype bovine torovirus (BToV), Breda virus, was identified in the faeces of calves with diarrhoea by electron microscopy in 1979, but never adapted to grow in vitro. The development of methods based on nucleic-acid amplification has allowed for the association of BToV to neonatal diarrhoea in several parts of the world, so that this virus should be taken into account in the diagnostic panels that are currently employed for the identification of enteric pathogens in calves.

Bovine enteric caliciviruses - are they relevant?

J. C. Bridger

Viruses resembling caliciviruses were observed over 30 years ago in association with calf diarrhoea, often in the same samples as established pathogens *(Woode & Bridger, 1978, J. Med. Micro.11, 441-452)*. In the UK, two viruses were described, now called Newbury1 and Newbury2. Infection of experimental calves with inocula freed of the known pathogens showed that they both caused diarrhoea but did not cross-protect *(Bridger, Hall & Brown 1984 Infect. Immun 43:133-138)*. They were refractory to further study until the genome sequence was obtained in the late 1990's.

Research over the last 10 years has shown that bovine enteric caliciviruses belong to 2 distinct genera in the family *Caliciviridae*. Viruses similar to Newbury2 are classified as noroviruses, the same genus as human noroviruses which are accepted as enteric pathogens of man. However, bovine noroviruses form a genogroup, genogroup III, distinct from human noroviruses *(Oliver et al., 2003. J. Virol. 77: 2789-2798)*. At present 2 genotypes / serotypes are recognized represented by the Jena virus from Germany (geno/serotype 1) and Newbury2 (geno/serotype 2) from the UK *(Oliver et al., 2006 J Clin Micro. 44:992-998)*. Viruses similar to Newbury1 form a previously undescribed new calicivirus genus distinct from the Vesivirus (Feline calicivirus, FCV), Lagovirus (Rabbit Haemorrhagic Disease virus, RHDV) and the Sapovirus genera. The name 'Becovirus' (or 'Nabovirus') has been proposed *(Oliver et al., Virology 350:240-250, 2006)*. Studies have shown that the diversity of the capsid protein is low suggesting one serotype *(D'Mello et al., 2009, Virology, 387, 109-116)*.

Both Newbury1 (Becovirus) and Newbury2 (Norovirus) induced diarrhoea and xylose malabsorption in gnotobiotic calves but Newbury1 produced more severe & consistent clinical signs. Newbury1 induced villous atrophy in the small intestine similar to rotavirus. Immunostained enterocytes were observed but they were few *(Hall et al., 1984 Vet Pathol 21:208-215)*. Bovine noroviruses have been identified in cattle throughout the world, including the UK, Germany, Netherlands, Belgium, US, Korea, New Zealand and Japan. Antibody prevalence is high with 78% and 84% of sera from calves and adults in the UK and Germany containing antibody to serotypes1 and 2 respectively *(Oliver at al., J. Clin. Microbiol.2007, 45, 3050-3052)*. Becoviruses are less well studied but have been found in Europe, North America and Asia. Seroprevalence studies have not been conducted.

Thus, the evidence indicates that enteric caliciviruses play a role in bovine enteric disease, as they do in enteric disease of man and pigs. The need to include them in prophylactic control measures requires assessment.

Bacteria associated with neonatal diarrhoea - a new perspective?

David GE Smith

Division of Bacteriology, Moredun Research Institute & Institute for Comparative Medicine, University of Glasgow

The main bacterial agents responsible for neonatal diarrhoea remain familiar, with Enterobacteriaceae *(E. coli and Salmonella)* still regularly implicated. The relationship between pathogens and their host(s) is complex and multifactorial, with many host and bacterial determinants being involved and *E. coli* represents a salient example. *E. coli* is a common resident (commensal) of the normal, healthy intestinal tract where it, indeed, may be an important component in promoting "normal" functions of the intestine. Characteristics that differentiate *E. coli* that can cause neonatal diarrhoea from "commensal" *E. coli* include many poorly-defined determinants as well as more familiar toxins, attachment factors (e.g. adhesins such as fimbriae) and key aspects will be summarised. The contribution of other facets – such as metabolic capabilities – are often overlooked and possible roles of novel characteristics will be appraised.

Parasites involved in neonatal diarrhoea

Arwid Daugschies

Institute of Parasitology, University of Leipzig

Calves are particularly susceptible to a variety of gastro-intestinal parasites. However, in Central Europe, helminth infections are relatively rarely observed in neonatal calves although nematodes may occur under keeping conditions that are compatible with parasite biology. For instance, *Toxocara vitulorum* and *Strongyloides papillosus* are transmitted to young calves via milk and may therefore occur in increased prevalence under respective keeping conditions but are of low prevalence in conventional herds. Strongylid infections are regularly found in yearlings kept on contaminated pasture. Calves are to be considered generally exposed and particularly prone during their first weeks of life to protozoan intestinal parasites such as *Cryptosporidium, Eimeria* and *Giardia*.

In general, *Cryptosporidium parvum* induces watery diarrhoea during the first 2 or 3 weeks after birth. Diseased calves shed tremendous amounts of oocysts that are immediately infective. Due to the high reproductive potential and short prepatent period of 2 to 14 days the infection is very rapidly and efficiently spread. Therefore within herd prevalence rates of 100 % can be expected under conditions that favour parasite distribution and survival (e.g. wet floor, poor hygiene, high stocking density, calving period). *C. parvum* is not very host specific and may cause zoonotic disease. Efficient control includes critical assessment and improvement of hygiene and keeping conditions. Cresol based products are suited for chemical disinfection, however, disinfection alone will not solve *Cryptosporidium* related problems. In such farms treatment with halofuginone will distinctly reduce the proportion of calves suffering from clinical cryptosporidiosis and oocyst excretion. It is important that treatment is initiated early enough and continued for 7 days to obtain sufficient protection

More than 20 *Eimeria* spp. have been described in cattle of which *E. bovis* and *E. zuernii* are particularly pathogenic in housed calves. Calves may acquire infection early after birth that may lead to disease starting at the end of the prepatent period of approx. 3 weeks or during patency. In case of clinical outbreaks it is rather typical that only a proportion of animals within a group are obviously affected and that the severity of scours varies in the diseased calves. Clinical coccidiosis may present in softening of faeces, watery dysentery or haemorrhagic diarrhoea, depending on the virulence of the pathogen, the susceptibility of the host and the infection dose. Treatment can be initiated immediately after the first case of coccidiosis has been observed in a group of calves. However, in this case some production loss and further contamination with oocysts can not be excluded. An alternative option is a metaphylactic treatment before onset of disease or oocyst excretion. Triazinones have been shown to be very efficient in such treatment regimes. However, this should be accompanied by critical assessment of hygiene and herd management.

Pathomorphology of the intestinal barrier in bovine neonatal diarrhea

Elisabeth M. Liebler-Tenorio

Institut f. Molekulare Pathogenese, Friedrich-Loeffler-Institut, Standort Jena, Naumburgerstr. 96a, D-07743 Jena, Tel: 49-364-804411, Elisabeth.Liebler-Tenorio@fli.bund.de

The intestinal barrier forms the largest contact area between body and environment. It is the target for viruses, bacteria and parasites causing neonatal diarrhea in calves. First, the different components forming the intestinal barrier (e.g. mucus, glycocalix, epithelial cells, cells of the intestinal immune system) will be reviewed. Then it will be discussed which components of the intestinal barrier are targeted, which lesions and which host reactions are induced by the different infectious agents causing neonatal diarrhea in calves. Gut-associated lymphoid tissues are an important part of the intestinal barrier. Their inductor and effector sites in the mucosa are essential for mucosal defence reactions. Distribution, morphologic characteristics and cellular composition in calves will be described

Experimental infection of antibody positive calves with bovine rota- and coronaviruses

Birgit Makoschey, Marina Timmermans, Petra Tonen and Ulrike Schmidt

**Intervet Schering-Ploug Animal Health, Wim de Körverstraat 35,
5831 AN Boxmeer, The Netherlands**

A number of different viruses, bacteria and protozoa can be associated with newborn calf diarrhoea. Co-infections with several pathogens are very common under natural conditions. Clinical disease has been reported after experimental infection of colostrum deprived calves with bovine rotavirus or bovine coronavirus, but not in calves that have been fed colostrum.

In the study reported here, calves that had been fed normal colostrum were infected with bovine rotavirus types P5G6 (n=4) and P11G10 (n=4) and bovine coronavirus (n=4) either alone or simultaneously with all three viruses (n=4). Virus excretion and clinical signs were compared between single and simultaneous infection. All animals had maternal antibodies against the virus(es) that were used for infection. Titers were comparable for the respective groups infected with a single or the three viruses.

All animals infected with a single virus survived until the end of the observation period. Most of them developed only very mild or no diarrhoea. By contrast, all four animals infected simultaneously with all three viruses had moderate or severe diarrhoea. Two of them had to be killed on humane reasons after episodes of severe diarrhoea. Both, rotavirus and coronavirus was detected in the faeces of all four animals infected simultaneously with the three viruses during several days. Moreover, both rotavirus G-types were present in a faecal sample from a co-infected calf.

From these data, it can be concluded, that the co-infection of calves with rota- and coronaviruses under laboratory conditions reflects the field situation, where co-infection of pathogens causes more severe disease than infection with single pathogens.

Moreover, the results reported here confirmed, that colostrum from unvaccinated dams did only partly protect against diarrhoea.

Neonatal calf diarrhoea: clinical diagnosis and sampling

Dr Yves Millemann

Pathologie du Bétail - ENVA - 94700 Maisons Alfort (France)
E-mail : ymillemann@vet-alfort.fr

Neonatal calf diarrhoea (NCD) 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. 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.

Clinical diagnosis

Calf scours is a clinical sign that is quite easy to diagnose. A precise diagnosis at individual level is not always indispensable, as it does not affect the course of actions to take but diagnosis is crucial at herd level as it has consequences in applied treatment or vaccination program.

NCD is generally observed together with other clinical signs, among them essentially dehydration along with acid-base imbalance.

NCD occurs mainly in calves less than six weeks of age, although calves up to four months of age may be affected. Different enteropathogenic agents are responsible for NCD at different ages and can act successively in the same calf. The age of the affected animal(s) is therefore crucial in orientating the suspicion diagnosis.

The aspect of diarrhoea can also help orientating the diagnosis, based on associated clinical signs, the observed systemic effects together with age of affected animals. For instance, a marked hypothermia may lead to a suspicion of colibacillosis, salmonellosis or coronavirosis, as well as a high mortality of affected calves. By contrast, a low mortality is rather evocative of rotavirosis. When NCD is accompanied by mortality, the practitioner can obtain interesting hints by realising a necropsic examination. Laboratory tests can be used to confirm pathogen suspected.

Paraclinical diagnosis

Clinical examination done on farm leads to a suspicion towards potentially implicated pathogens. Several laboratory tests are available to the practitioner to confirm the diagnosis. Calves faeces or intestinal content are the most frequent samples taken in case of suspicion of enteropathogen implication in calf diarrhoea. Different exploration methods are available, ranging from microscopic examination, antibiotic resistance search, Elisa technique to real-time PCR for identification of agents involved.

However, other samples such as naso-pharyngeal lavage, blood sampled on live calves or lung, liver, spleen, lymphatic nodes samples taken on dead calves can be used for identification of agent implicated in calf diarrhoea. Some commercial available tests can also be used on faeces, blood or colostrum samples: they have the great advantage of providing results within minutes which make them very useful in farm situation.

Moreover, when faced to an outbreak of NCD in a herd, the veterinarian practitioner must check FPT by measuring gamma-globulinemia in calves and look for other risk factors that contribute to the development of the outbreak.

References

Frémont A, Cornuejols MJ, Couquet C. 2004. Enquête épidémiologique sur les diarrhées néonatales.
Point Vétérinaire spécial « Actualités en pathologie digestive des bovins », 20-23.
Lundborg GK, Svensson SC, Oltenacu PA. 2005.
Herd-level risk factors for infectious diseases in Swedish dairy calves aged 0–90 days Prev Vet Med, 68(2-4), 123-143.
Maillard R. 2006a. Composition et rôle du colostrum chez les bovins.
Point Vét; 37(Reproduction des ruminants : gestation, néonatalogie et postpartum) 106-109.

Laboratory Diagnosis of Neonatal Calf Diarrhoea

David Snodgrass

Biobest Laboratories Ltd, 6 Charles Darwin House
The Edinburgh Technopole, Milton Bridge
Nr Penicuik, EH26 0PY, Scotland

Diarrhoea in neonatal calves is a complex syndrome; it can be caused by a variety of viruses, bacteria and parasites; their interaction with the calf is complex and frequently subclinical; and factors influencing the clinical outcome are physiological, immunological, nutritional and environmental.

Faeces are the most readily-available and common material available for diagnosis, although faecal content may only partially reflect the pathogens present in the small intestine. Most enteric pathogens are excreted for relatively short periods and may be absent in faeces after a few days illness. The rapidity of post-mortem change in the intestine minimises the value of histopathology in routine diagnosis.

The challenge is to reach a herd as well as individual diagnosis. Appropriate laboratory techniques and their interpretation are described.

On-site diagnosis of cryptosporidiosis and giardiasis in calves

Thomas Geurden, Jozef Vercruysse, Edwin Claerebout

Laboratory of Parasitology, Faculty of Veterinary Medicine, Ghent University

Cryptosporidium parvum is considered as the most important enteric pathogen in calves until the age of one month. *C. parvum* infection can lead to a profuse and watery diarrhea typically in calves aged between 5 and 28 days. From the age of one month, calves reared indoors can be affected by *Giardia duodenalis*. Recent data confirm the pathogenic importance of *G. duodenalis* in housed calves, causing chronical diarrhea, ill thrift and retarded growth.

Cryptosporidiosis or giardiasis in calves can be suspected based on clinical signs, data on farm management and the exclusion of other infectious diseases, such as coccidiosis. The clinical diagnosis needs to be confirmed by the detection of the parasite in faecal samples. Microscopical examination (ME) of stained faecal samples for *Cryptosporidium* oocysts or *Giardia* cysts is a widely used diagnostic technique, but requires trained microscopists. Immunological techniques to detect (oo)cyst antigens in the faeces, such as immunofluorescence assays (IFA) and ELISA, are believed to be more sensitive and more specific than microscopy. More recently, Polymerase Chain Reaction (PCR) has been shown to be very sensitive and specific. However, these tests can only be performed in well equiped laboratories. Reliable, fast and easy-to-use diagnostic tests to detect *Cryptosporidium* and *Giardia* infections on-site might provide a practical and time saving alternative. New immunochromatographic assays (dipsticks) are now available for use in calves, but their diagnostic properties have not yet been validated. Assessment of the sensitivity and specificity of diagnostic tests for *Cryptosporidium* and *Giardia* is hampered by the lack of reliable gold standards, leading to over- or underestimation of the performance of new tests. Therefore in the present studies, a Bayesian statistical approach was used to circumvent the need for a gold standard. As the properties of a diagnostic test can be different in calf populations with different (oo)cyst excretion patterns, and can not be extrapolated from one population (e.g. healthy calves in a prevalence study) to another (e.g. calves with diarrhea), the different diagnostic tests were validated in different calf populations, including calves with diarrhea that were suspected of having clinical cryptosporidiosis or giardiasis.

In faecal samples from an epidemiological study, Bayesian analysis indicated that PCR assays had a higher sensitivity than all the 'conventional' diagnostic techniques to detect Cryptosporidium (ME, IFA and two ELISAs) and that the specificity of the immunological assays and PCR was high (89-95%) compared to ME (79%). In calves with diarrhea, immunological assays were found to be specific (IFA: 95%; Tetra ELISA: 96%; Techlab ELISA: 93%; dipstick: 92%) and sensitive (IFA: 97%; Tetra ELISA: 94%; Techlab ELISA: 95%; dipstick: 88%). Despite a slightly lower sensitivity, the dipstick assay thus provided a practical alternative to laboratory diagnosis of clinical cryptosporidiosis in calves.

ELISA (Se 89% and Sp 90%) was found to be a sensitive and specific diagnostic technique to detect *Giardia* infections in epidemiological settings, whereas IFA (Se 77% and Sp 95%) and ME (Se 56% and Sp 87%) were less sensitive. In calves with diarrhea, preliminary data indicate that dipstick assays are less sensitive compared to IFA to detect *Giardia* copro-antigens.

Since *C. parvum* oocysts and *Giardia* cysts are immediately infective upon excretion and can survive for several weeks or months outside the host, control measures must aim to reduce or prevent (oo)cyst transmission, by combining treatment with management measures and the use of suitable desinfection procedures to destroy infective oocysts. Halofuginone is registered in several countries for the preventive and therapeutic treatment of cryptosporidiosis in calves. However, no registered drugs are available for treatment of *Giardia* infections in calves. In experimentally infected calves, treatment with paromomycin, fenbendazole or albendazole reduced the peak and the duration of *Giardia* cyst excretion. Fenbendazole treatment also resulted in a clinical benefit and an increased weight gain. In naturally infected calves with clinical giardiasis, fenbendazole treatment combined with cleaning and disinfection of calf housing resulted in a near to 100% reduction of *Giardia* cyst excretion up to 4 weeks after treatment. No clinical signs of giardiasis were observed after treatment and no adverse reactions to the fenbendazole treatment were recorded.

Risk factors for neonatal diarrhea in beef calves

Dr Fatah Bendali

Institut de l'élevage, Paris, France

Diarrhea is the most commonly reported disease of calves and the major cause of neonatal mortality. The incidence rates was reported around 20%. In the Midi-Pyrénées region (France), it was estimated that 80% of herds and 20% of neonatal calves were affected by diarrhea.

The prevention of neonatal diarrhea in beef calves is difficult due to the large number of etiological agents and should be centered around management factors and to determine the relative importance or priority of each the above factors.

This study is different from those previously reported in the literature because it was carried out prospectively with a large randomly selected sample population of beef calves, in addition, an original statistical model was used to estimate the relationships between both individual and herd-level risk factors and the occurrence of diarrhea.

Materials and methods

We carried out a prospective study on a random sample of 94 herds. All calves born during the period were enrolled and followed during the first month. The farmer recorded all data relating to calving and care of the newborn or dam information and each event related to calf health (diarrhea..). Calves that were less than 1 week of age at the time of the visit were sampled for serum Total Protein(TP). A questionnaire was given to determine herd management practices (housing, feeding...).

Descriptive statistics and frequency distribution variables were performed using SAS®. Because data from animals within the same herd are not independent, SUDDAN® software was used. The analysis was based on Cox's proportional hazards model to estimate parameters.

Analysis was achieved using 3 steps. interrelationships of all variables taken individually with the occurrence of diarrhea were tested in a univariate model. Then, any variable with $p \leq 0.2$ was eligible for the next step in a multivariate model where they were grouped within categories (feeding, management, calving, housing...). This grouping procedure took into account potential confounding factors. In final multivariate model was fitted with all variables which had remained significant during the two previous steps.

Results & discussion

A total of 3157 calves were followed. General incidence of diarrhea was 14.6%.

From practices related to calving, several factors were associated with diarrhea. Dystocia increased the risk of diarrhea by 1.44 relative risk. It causes stress for the newborn calf, reduced calf vigor and delayed ingestion of colostrums, and calves may be recumbent for longer period.

Calves with dyspnea at birth (7%) required more attention and had 1.85 high risk of diarrhea.

Colostrum factors were not associated with the occurrence of diarrhea in our study. One explanation may be that the majority of calves (76%) have a sufficient level of total serum protein (>50 g/L). Indeed the average concentration in 1174 calves was 55.48 g/L. The frequency of feeding colostrum in our study agrees with the above mentioned factors, but was not statistically associated (RR=1.41 and p=0.12) in the final model. There was no relationship between treating the navel and the occurrence of diarrhea. 22% of calves were born from primiparous cows, parity was not associated with diarrhea.

High ammonia concentration (RR=1.46, p=0.08), this result was observed in 7.4% of herds, and could be a consequence of a bad ventilation or insufficient quantities of straw.

Cleaning after each calving season decreases the risk of diarrhea (RR=1.92, p=0.006). Furthermore, when cows are dirty, their calves are logically more at risk of illness(RR=1.45, p=0.05). Cleaning before the calving season was associated with an increased incidence of diarrhea (RR=0.54, p=0.009).

Calf stocking density and overcrowding was significantly associated with morbidity, and was estimated to be 1.74 times more than for calves with sufficient space.

December was a high risk month (RR=2.74, p=0.04) probably due to weather and stressed calves, but also due to environmental pathogens agents like E. coli. The highest risk was observed in March (RR=4.12, p=0.0004). It could also be explained by calf overcrowding and accumulation of manure.

The results illustrate that calves born in non vaccinated herds have 2.11 more risk of diarrhea. In addition, at the 'herd level', the analysis showed that dam vaccination against agents like BVD, Clostridium perfringens, but not against E. coli, rotavirus and coronavirus, seems to decrease the risk of diarrhea (RR=2.01, p=0.03). The finding of a negative association between dam vaccination with E. coli, and calf diarrhea was unexpected. Offering minerals and vitamins to cows during the dry period were associated with decreased diarrhea (RR=1.56, p=0.07).

Results showed that calves from herds with no concentrate feeding of concentrate during the dry were at higher risk of illness (RR=1.57, p=0.06).

Herds with high incidences of diarrhea in the previous calving season were more likely to reproduce this high rate and calves had nearly 2 times (RR=1.92) more risk of diarrhea.

Various additional factors reported in the literature were not associated with diarrhea in our study (feeding colostrum, navel dipping, calf location, parity). The similar nature of some management practices (homogeneity between herds) may contributed to the lack of identification of other significant herd-level risk factors.
We feel that the majority of risk factor values varied between 1.5 to 2. This indicate that it is not easy to identify separately individual effects of several management practices, and very often, many factors are associated. That consequence is that providing advice to farmers should take into account several parameters simultaneously and under a priority and strategy.

Pathogens in faeces of calves aged from 1-21 days in Dutch dairy herds; Prevalence and risk factors

Menno Holzhauer[1], Chris JM Bartels[1], Theo Lam[1], Jeroen van de Ven[2] and Ton de Gee[1]

[1] **GD/AHS, Deventer, The Netherlands**

[2] **Intervet Schering-Plough Animal Health, Brussels, Belgium**

In spring 2007, over 400 calves less than 22 days of age, from 108 different Dutch dairy farms, were sampled to estimate the prevalence of non-normal faeces ('custard-like' or 'diarrhoea') and the presence of intestinal pathogens in de faeces. The investigated intestinal pathogens were: *Eschericia coli K99 (E. coli)*, Corona virus, *Cryptosporidium parvum (C. parvum)*, Rota virus and *Clostridium perfringens (C. perfringens)*. In addition, through clinical examination and use of a questionnaire, information was collected on animal characteristics (e.g. age, sex, temperature) and herd management (e.g. housing, colostrum and milk supply). The relation between intestinal pathogens and faecal consistency were analysed using a multinomial regression analysis, whereas putative risk factors related to the presence of each of five pathogens were analysed using logistic regression with random effects.

Preliminary results have shown that 57 percent of calves had faeces of normal colour (brownish) and consistency (firm), 24% of the calves had 'custard-like' faeces (yellowish-coloured with custard consistency) and 19% of calves had 'diarrhoea' (watery-like faeces). E. coli was detected in 2.6% of calves, Corona virus in 3.1%, Rota virus in 17.7%, *C. parvum in* 27.8% and *Cl. perfingens* 54.0% of calves. *E. coli* was present in 10 herds (9%), Corona virus in 13 herds (12%), Rota virus in 50 herds (46%), *C. parvum* in 62 herds (57%) and *Cl. perfingens* in 92 herds (=85%). *E. coli* and Corona virus were detected in only 1 or incidentally in 2 calves per herd, whereas *C. parvum* and *Cl. perfingens* were frequently diagnosed in up to 4-5 calves per herd. Of the pathogens, only *C. parvum* was related to 'custard-like' faeces whereas for 'diarrhoea', all pathogens except Corona virus were related. Preliminary results about risk factors for the presence of the different pathogens will be discussed at the conference.

Rotavirus and concurrent infections with other entero-pathogens in neonatal diarrheic dairy calves in Spain

García, A.

Departamento de Producción animal, Sanidad Animal y Ciencia y Tecnología de los Alimentos, Facultad de Veterinaria, CEU - Universidad Cardenal Herrera, Avda. Seminario s/n, 46113 Moncada (Valencia), SPAIN
Tel. 34 96 1369000 ext. 1223, Fax 34 96 1395272
E-mail angel@uch.ceu.es

Faeces samples from 218, one to 30 days old, diarrheic dairy calves in 65 dairy herds were screened for the presence of rotavirus and concurrent infections with coronavirus, Cryptosporidium, F5+ Escherichia coli and Salmonella spp. Calves were grouped according to their age as follows: 1-7, 8-14, 15-21 and 22-30 days. Rotavirus infection was detected in 46.9%, 45.6%, 33.8% and 48.3% of the calves in the respective age-groups. No significant differences in the detection rate of rotavirus were found among calves on the different age-groups. Rotavirus was the only enteropathogen detected in 39 of the 93 (41.9%) diarrheic calves positive to this agent. Concurrent infections with other enteropathogen(s) were detected in 31.3%, 33.3%, 20.6% and 3.4% of the rotavirus infected calves in the age-groups 1-7, 8-14, 15-21 and 22-30 d, respectively. A significant age-associated decrease in the detection rate of mixed infections ($p < 0.01$) was found. The detection rates of the other enteropathogens considered in calves with rotavirus infection were 20.4% for coronavirus, 85.2% for Cryptosporidium, 16.7% for F5+ E. coli and 1.8% for Salmonella.

Epidemiology of neonatal diarrhea in dairy and beef calves in the czech republic

Illek J.

Clinic of Ruminant Diseases, Faculty of Veterinary Medicine, Veterinary and Pharmaceutical University Brno, Czech Republic

Diarrheal diseases in calves in the early postnatal period are the most significant problem in this production stage and are responsible for considerable direct and indirect losses. The occurrence of diarrhea is high and it affects 10 – 90 % calves in the first three weeks of life, depending on many factors. Usually, mortality ranges from 3 to 10%, but in severely affected herds it can be over 30%. In the Czech Republic, calf losses range between 10 and 12 %, and are caused mainly by diarrhea (72-76% of total losses). SZENCI (2009) has reported 10.5 % calf losses in Hungary, with diarrhea being the most frequent cause of death. In Poland, calf losses range between 10 to 15 % (Nicpoň, 2009).

The aetiology of neonatal diarrhea includes a wide range of factors, from dietary and management failures to infections with various pathogens. The non-infectious diarrhea (non-specific scours) is caused by management mistakes most frequently such as poor colostrum and milk replacer management, housing and hygiene. However, the infectious diarrhea occurs much more often. It develops in calves weakened due to dyspepsia or as a primary disease under the conditions of poor hygiene, insufficient colostral immunity and high infectious pressure on farms with high density of animals.

The main causes of diarrhea are mixed viral, bacterial and protozoan infections. In the 16 herds under study (12 dairy and 4 beef herds), faeces from 120 calves suffering from diarrhea were examined for the main pathogens – rotaviruses, coronaviruses, E. coli (ETEC and EPEC) and cryptosporidia *(Cryptosporidium parvum)*.

In 52 % calves examined, mixed infections with rotaviruses, cryptosporidia and ETEC were diagnosed. In 8% calves, mixed rota and coronaviral infections were diagnosed, in 7% calves only rotaviruses and in 6% only EPEC were found.

In 5 problem herds with diagnosed mixed infection, the effect of vaccination of cows in late pregnancy with the Rotavec Corona vaccine was monitored. Their calves showed significantly better health status, the occurrence of diarrhea was very low and deaths were scarce. The calves from non-vaccinated mothers suffered from neonatal diarrhea and mortality ranged from 10 to 36% among the herds under study.

By further monitoring of Holstein calves it was demonstrated that feeding the calves with Kolostran (feed containing dry colostrum and probiotics) favourably influenced their health.

Right after the birth, the calves were orally administered the Kolostran paste. Kolostran had a favourable effect on the immunity of calves and serum immunoglobulin concentrations. Very good results were achieved in beef calves because a high percentage of calves in feedlot do not receive a sufficient amount of colostrum and show hypogamaglobulinaemia much more frequently than dairy calves.

In order to achieve the efficient prophylaxis of diarrhea in neonatal calves, the approach must be comprehensive and must include optimum nutrition of the cow in the close-up period, effective vaccination against the main pathogens, correct calving management and care of the newborn calf, early administration of a sufficient amount of colostrum and high level of hygiene in the calving pen and individual calf housing.

The study has been elaborated within the project no. QH71156 of the Czech National Agency for Agricultural Research (NAZV).

Impact of frequent calf diseases on subsequent productivity

Martin Kaske

Clinic for Cattle University of Veterinary Medicine,
D - 30173 Hannover, Germany
martin.kaske@tiho-hannover.de

Successful replacement of heifers is crucial for the profitability of dairy herds. An age at first calving of 24 months requires mean daily weight gains between birth and insemination of roughly 800 g. Such an intensive calf rearing presupposes the successful prevention of hazardous diseases. Acute undifferentiated diarrhea and the enzootic pneumonia complex represent the two major diseases of concern. At first glance, their impact on productivity is caused by fatalities, reduced weight gains during the acute disease, increased expenses for veterinary service as well as pharmaceuticals and the increased expenditure of time for the fostering of ill calves. However, reduced weight gains after apparent recovery may contribute significantly to economical losses. Moreover, effects of a calfhood history of being treated for diarrhea, umbilical infections and pneumonia on survival and age at first calving have been evaluated in several studies. In most of these studies, clear detrimental effects of calf diseases on further performance were demonstrated. In some papers, however, calfhood morbidity was not significantly associated with subsequent productivity. These inconsistent results are a consequence of the fact that both diarrhea as well as pneumonia are multifactorial diseases. The variety of infectious agents residing in the barn, the abiotic environment and management factors determine on the one hand the incidence, severity and duration of diseases. On the other hand, these factors play a pivotal role for the consequences of a recent disease which aggravates general statements in respect to the effects on subsequent performance. However, there is general agreement that by optimization of the key components for successful rearing of calves (in particular feeding, housing and hygiene) the incidence of diarrheic episodes and respiratory diseases can be minimized. Concomitantly, dairy calves achieve daily weight gains far beyond those obtained on typical commercial dairies. Overall it is concluded that the prevention of calf diseases represents an important precondition for a subsequent resilient health status and a sustainable high productivity.

Neonatal Calf Health Management

Keith Cutler BSc BVSc DipECBHM MRCVS

Endell Veterinary Group, 49 Endless Street, Salisbury, Wiltshire, SP1 3UH

The birth of a calf is always an exciting event. It is the culmination of significant investment in cow care, fertility management and genetic selection. It represents the entire output of a beef suckler cow and the future for a dairy enterprise. Care of the newborn calf deserves, therefore, the highest priority and yet how frequently is this seen in modern farming systems?

It could be argued that care of the neonatal calf begins well before birth. Cows need to be in a 'fit, not fat' body condition to minimise problems at calving and to ensure the production of good quality colostrum. Herd health status should be considered and managed since many infectious diseases, BVD, leptospirosis and neospora for example, can infect the foetus in utero and have consequences for the health of the newborn calf. Specific mineral deficits, particularly iodine, may also need to be addressed.

Once born the calf needs a clean and comfortable environment. Indoors, a well ventilated airspace (without being draughty) and a dry bed are vital. Outside there will need to be sufficient shelter from inclement weather. For dairy calves reared away from their dam, consideration should be given to the potential health risks which may be associated with feeding waste milk and how these can be managed. Feeding powdered milk replacer may eliminate many of these risks but introduces further dilemmas; should a skim- or a whey- based powder be used?, should it be fed hot or cold?, should it be fed once a day, twice a day or ad lib? High quality concentrate feed and roughage and potable water should also be provided.

Infectious disease management is particularly important to ensure the health of neonatal calves. Since antibodies cannot cross the bovine placenta, calves rely entirely on an early intake of colostrum to gain passive immunity from their dam. It is frequently quoted that newborn calves should receive six pints, three litres or 10% of their bodyweight of good quality colostrum within six hours of birth and a further similar amount before reaching one day of age. If either intake or colostral quality is suspect, action should be taken to remedy the situation without delay.

The navel, before it dries and heals, is particularly vulnerable to infection which may result in both localised or systemic problems. Topical application of an astringent and antiseptic preparation, strong iodine BP for example, soon after birth, and again twelve hours later if the dam licks the initial application off, is recommended.

The most common health issue affecting neonatal calves is diarrhoea. This has a multifactorial aetiology and one or more of a wide variety of infectious agents may be involved. Treatment priorities should focus on correcting fluid and acid-base balance using appropriate oral and, if necessary, intravenous fluids and on nursing before addressing the specific pathogens involved, if this is, indeed, possible.

Colostrum Management

Juan V. González-Martín DVM, PhD, Dipl ECBHM

Clinic for Ruminants, Veterinary Faculty, Complutense University of Madrid.
TRIALVET Veterinary Consultancy and Research SL
(Web: www.trialvet.com and e-mail: juanvi@vet.ucm.es or trialvet@gmail.com)

Introduction. Neonatal morbidity and mortality, although important, are not the only interest of a good colostrum management. Postweaning health, especially respiratory problems, growing rate, feed efficiency, age at first calving, milk production, and culling must be focused in order to detect when it is necessary to improve colostrum management in farms with a low level of neonatal scour problems.

The management of the cow. Related to dry cow management, a dry cow period 45-60 days long is recommended. Producers should feed dry cows with balanced rations and could be interesting to inject selenium and vitamin E in late pregnancy. The vaccination of the dam against neonatal diarrhoea pathogens increases colostral antibody presence against *E. coli*, rotavirus and coronavirus. Colostral volume decreases due to different causes as heat stress, age of the dam and breed. Related with the two last issues we can do very few. First milking should be performed in the two hours (max. six hours) after calving, and it should be avoid the pooling of colostrum and the use of colostrum from mastitic, watery, bloody or from cows that leaked before calving.

Colostrum management. Colostrum is not only IgG1, others Ig, B cells, CD cells, macrophages, neutrophils, growing factors, hormones, cytokines, lactoferrin, lysozyme, lactoperoxidase , nutrients and probably other components unknown are also present. These components work at local and systemic level. Probably, when colostrum begin to the own calf's mother and it's not contaminate, overheated or frozen; we will reach the best results. A good idea will be the classification of colostrum in order to use the best one for the first meal and the others for successive ones. Frozen, refrigerated or pasteurized (60ºC/60minutes) colostrum could be an acceptable option to solve different herd problems.

The management of the calf. Before calving underfeeding of the dam and heat stress originate low weight calves. At calving, neonatal acidosis mainly caused by dystocia is probably the most important issue. Edematous head (mainly the tongue) and delay to stand up are the basic characteristics for its diagnosis. Also cold is an etiology of postnatal acidosis, and even in a low grade it can worsen neonatal acidosis. Four litres of colostrum at body temperature should be administered to the calf by a nipple bottle in the two hours after calving (max. six); this is probably the best option. If possible continue administrating two litres each twelve hours for tree days. When the calf does not collaborate the use of an oesophageal feeder is a good option. Oral treatment with any type of oral products before or at the same time that colostrum, could disrupt immunoglobulins mechanism of absorption; and thus it should be avoid. We should consider that the calf does not only births without specific immunity, but also it suffers a severe immunosuppressant effect due to the effect of partum corticosteroids lasting until five days post calving. For this reason, we must give colostrum in adequate amount, quality and time, and also reduce as much as possible stress causes (I.e. cold, absence of visual contact to other calves...). In general, we must avoid any procedure that could produce stress. Practices as very early vaccination could be counter producing.

Assessing the process. The colostrum could be tested by the use of a colostrometer (specific gravity >1.050) and by total bacteria count (<100,000 cfu/mL) and total coliform count (<10,000 cfu/mL). The immunoglobulin level in the calf could be tested by serum refractometry (>5.5 g/dL).

Prevention and Treatment of Cryptosporidiosis in Calves - Special Emphasis on the use of Halofuginone Lactate in Portugal

L.M. Madeira de Carvalho[1], S. Martins[2], S. Sousa[3], J. Bacelar[2], J. Cannas da Silva[4,5]*

[1] Interdisciplinary Research Center on Animal Health, Faculty of Veterinary Medicine, Technical University of Lisbon, Av. Universidade Técnica, Pólo Universitário do Alto da Ajuda, 1300-477 Lisbon, Portugal.
[2] Cooperativa Agrícola do Bunheiro, Murtosa, Portugal.
[3] Escola Universitária Vasco da Gama, Laboratório de Parasitologia, Coimbra, Portugal.
[4] Laboratório de Medicina Veterinária, Ltd., Santarém, Portugal.
[5] Intervet-Schering-Plough Animal Health Portugal, Ltd.

***Corresponding author: Luís Carvalho: madeiradecarvalho@fmv.utl.pt**

Cryptosporidium parvum is now recognized as one of the leading causes of diarrhea in pre-weaned dairy calves up to 12 weeks of age, being a common infection on dairy cattle farms in Portugal. The prevalence of this coccidian may range from 12% to 75% of infected animals per farm and a recent study carried out in the Northwest of Portugal showed 100% positive farms.

Therefore, it is important that both practitioners and farmers consider cryptosporidiosis as an important agent in what concerns calf health, as well as an important factor for differential diagnosis when investigating the aetiology of scouring in calves under 1 month of age. Infected calves are responsible for high levels of environmental contamination by excreting large amounts of oocysts in their faeces, being considered the primary source of *Cryptosporidium* in dairy herds.

Attention is needed to reduce the frequency and intensity of this infection in dairy herds not only because of its economic impact but also because is becoming an increasing concern to public health. Prevention of *C. parvum* infection relies on good management practices. Calves should be born and raised in a clean, dry environment and should be housed on individual hutches or boxes. Healthy calves should be confined separately from sick calves, and these should be cared by different people, using different equipment. Calf rearing areas should not be occupied continuously and should be thoroughly cleaned between batches of calves.

Therapeutic measures must associate agent-specific drug, like halofuginone lactate (Halocur)®. The use of this drug in field trials in Northwest Portugal has shown that its administration *per* os at 1-2 days after birth during one week has a significant effect on reducing oocyst shedding and diarrhoea at 14 days of age. The oocyst excretion in the control group peaks on Day 7 (100% of the animals) and Day 14 (94%), but was lower for the same periods in the halofuginone group (30,5% and 55,6% of the animals, respectively), being the intensity of shedding significantly lower, as well as the number of calves presenting diarrhea on Day 14. Therefore, a combination of good hygiene measures associated with a strategic administration of halofuginone lactate contributes to decrease Cryptosporidium environmental contamination, the zoonotic risk of cryptosporidiosis, together with the improvement of animal welfare.

Enhancement of Passive Protection Against Bovine Enteric Coronavirus Using Rotavec Corona™

Colin F Crouch

Intervet Schering-Plough Animal Health, Milton Keynes, UK

Neonatal calf diarrhoea is a complex disease associated with a number of infectious agents occurring either singly or in combination. Economic losses are suffered as a result both of mortality (which can typically range between 0 and 80%) and also veterinary costs and decreased productivity of the survivors.

There is little comprehensive information available on the prevalence of the recognised causative agents of diarrhoea in the newborn calf; however the majority of studies indicate that rotavirus is the most common pathogen followed by *Cryptosporidium parvum*, coronavirus and enterotoxigenic *Escherichia coli* (ETEC). Rotavirus, coronavirus and *C. parvum* are ubiquitous in the environment and as a result infection of the neonate is extremely common. In addition to causing clinical disease in neonates, these agents may also be associated with subclinical disease in adult animals, who may therefore act as reservoirs for re-infection.

Two approaches have been taken in the development of vaccines for the protection of neonatal calves against rotavirus and coronavirus infection. The first approach involved oral infection of the calf with live attenuated virus to stimulate active immunity. Whilst laboratory studies initially confirmed that the incidence of diarrhoea in neonatal calves vaccinated with attenuated rotavirus was reduced, the vaccine was not found to be effective in blind field trials. The second approach involves the enhancement, by vaccination of the dam, of passive protection provided to the young calf through the ingestion of colostrum and milk. This has been shown to be highly effective in the field and is well established for protection against rotavirus and ETEC infections of calves. Efficacy against bovine enteric coronavirus has however been more difficult to establish, with numerous publications indicating that some vaccines were even unable to demonstrate significant seroconversion following vaccination. More recent vaccines, such as Rotavec Corona™ have demonstrated not only significant increases in the mean specific antibody titre in the serum of vaccinated animals, but an accompanying increase in protective antibodies in colostrum and milk for at least 28 days post calving. The reasons for such enhanced responses are probably related to careful optimisation of the adjuvant and the antigenic load employed in the vaccine. Optimal stimulation of immunity has also resulted in a wide vaccination window offering increased flexibility to the farmer for whole herd vaccination.

Notes

EARLY CARE, LIFE LONG GAINS

Intervet International bv
P.O. Box 31, 5830 AA Boxmeer
The Netherlands
Phone +31 (0)485 587600
Fax +31 (0)485 577333
E-mail info@intervet.com
www.intervet.com

R97F'09

Intervet
Schering-Plough Animal Health

Université de Liège