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This form is in Word format and the boxes may be expanded or reduced, as appropriate.

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<table>
<thead>
<tr>
<th><strong>Project identification</strong></th>
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<tr>
<td>1. Defra Project code</td>
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<tr>
<td>2. Project title</td>
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| 3. Contractor organisation(s) | Veterinary Laboratories Agency  
Woodham Lane  
New Haw  
Surrey  
KT15 3NB |
| 4. Total Defra project costs (agreed fixed price) | £ 401,684 |
| 5. Project: start date ............... | 01 April 2009 |
|                           end date ............... | 31/03/2011 |
6. It is Defra’s intention to publish this form.
Please confirm your agreement to do so. ........................................... YES ☒ NO □

(a) When preparing SID 5s contractors should bear in mind that Defra intends that they be made public. They should be written in a clear and concise manner and represent a full account of the research project which someone not closely associated with the project can follow.

Defra recognises that in a small minority of cases there may be information, such as intellectual property or commercially confidential data, used in or generated by the research project, which should not be disclosed. In these cases, such information should be detailed in a separate annex (not to be published) so that the SID 5 can be placed in the public domain. Where it is impossible to complete the Final Report without including references to any sensitive or confidential data, the information should be included and section (b) completed. NB: only in exceptional circumstances will Defra expect contractors to give a “No” answer.

In all cases, reasons for withholding information must be fully in line with exemptions under the Environmental Information Regulations or the Freedom of Information Act 2000.

(b) If you have answered NO, please explain why the Final report should not be released into public domain

Executive Summary

7. The executive summary must not exceed 2 sides in total of A4 and should be understandable to the intelligent non-scientist. It should cover the main objectives, methods and findings of the research, together with any other significant events and options for new work.

This report describes the epidemiology, pathology and virology of bovine neonatal pancytopenia (BNP) in young calves in Great Britain. All cases were reported and enrolled via a VLA or SAC surveillance laboratory.

Each investigation examined the same cohort of calves, but not all tests or examinations were carried out on every animal and the numbers varied between reports. Between May 2009 and September 2009, to establish a case definition, epidemiological exploration was done on 75 calves, histopathology was carried out on 89 calves, haematology was done on 30 cases and 54 cases were examined for evidence of primary virus infection.

The cases were distributed throughout England and Scotland, with relatively few cases reported in Wales. Generally, one to four cases were reported per farm (median = 1), but a few farms reported >5 cases.

The condition BNP appears to have emerged slowly through 2007 and 2008 and increased in frequency in 2009 and no patterns suggesting a point source introduction were observed. The incidence is still low and the small number of calf deaths attributed to this condition has not yet made a detectable contribution to the overall mortality among young calves in the national herd (RADAR data).

A case-series study was carried out in 2009 on 75 case calves. Enrolment criteria for the case-series study were: young calves less than 30 days old with either clinical features of unexplained / unexpected haemorrhage or necropsy findings of unexplained / unexpected haemorrhage at one or more sites. Calves were excluded if Bovine Viral Diarrhoea Virus (BVDV) RNA was detected in spleen or other obvious reasons could explain the bleeding. At least one calf on each case farm had been subject to full necropsy and characteristic bone marrow changes identified by histopathology.

The herds of origin of the cases in the case–series study were larger (mean = 386) than the national average (mean = 112). It is unknown whether this is a characteristic of the disease, associated with the cause or a characteristic of the Veterinary Laboratories Agency (VLA) / Scottish Agricultural Colleges (SAC) client base.

A small group of husbandry factors applied to >60% of the case calves in the case-series study and these were investigated further in a case-control study. These factors included: received colostrum (99%) mainly from own dam (91%); indoor housing of case calves (75%), sheep present on farm (63%), grass feeding
of dam during or after pregnancy (79%, 86%) and buying in of new cattle (67%).

Furthermore, all dams had received at least 1 vaccine during the previous 12 months before calving or in the 4 weeks post calving 97% of the dams had been vaccinated against BVDV, 63% against leptospirosis and 88% against Bluetongue virus. Because of the high immunisation load, it was not possible to separate the effect of the different immunisations.

A case-control study was conducted in 2010 using 56 case and 58 control calves. Enrolment criteria for this study were: a case was defined as a calf younger than 29 days with unexplained haemorrhages detected at necropsy and characteristic bone marrow pathology. For each case a control calf was matched by purpose of herd of origin (e.g. dairy or beef) and was from a herd with no history of BNP.

Risk factors identified in the case-control study included: the use of the BVDv vaccine PregSure in the dam at any time from the start of 2005 through to the birth of the calf, (Odds Ratio (OR) 40.78) the herd being located in Scotland (OR 9.71), the presence of sheep on the farm, and housing of the calf (OR 0.11).

Histological examination was carried out on 346 calves within the case definition until 28/02/2011. Of these, 333 were characterised by reduction of all three main blood cell types in the bone marrow (trilineage hypoplasia), (“aplastic anaemia”) (Group A). Eight calves were classified as Group B based on presence of diffuse regenerative responses or no significant change. Four Group C calves exhibited late erythroid and megakaryocyte bilineage hypoplasia (marked reduction of two blood cell types, namely red blood cells and thrombocytes). One calf had changes intermediate between those of group A and group C.

The large majority of calves meeting the case definition had histological lesions of trilineage hypoplasia (TLH). Trilineage hypoplasia is an end-stage lesion and histological evidence of a causative agent was not detected at the time of examination. Thrombocytes, which are produced by megakaryocytes, are essential for normal blood clotting. The megakaryocyte loss (detected in calves in groups A and C) therefore will have resulted in lack of thrombocytes (thrombocytopenia) and abnormal bleeding.

The clinical signs of BNP appeared on average around day 12 of age, but range from day 0 to day 20. The majority of calves die within a few days of showing clinical signs. Calves with a histopathology classification of B or C were more likely to die and show clinical signs at a very young age (mean=4), whereas calves with histopathology classification A showed clinical signs around day 13 of age. No calves classified in group A were reported to show clinical signs before day 2 of age.

Three patterns of clinical signs were reported. The majority of calves showed some unexpected bleeding combined with systemic signs such as pyrexia, malaise, respiratory signs or diarrhoea. A small group exhibited abnormal bleeding without any signs of general effects. And a third group presented as sudden death with no evidence of external bleeding.

A number of older cattle with extensive bleeding were examined also. None had lesions of trilineage hypoplasia. There is no evidence that BNP is occurring in cattle older than one month in the UK.

Haematological examination of live affected calves (cases) revealed a characteristic loss of three blood cell types (trilineage), namely white blood cells, red blood cells and platelets, giving rise to the term pancytopenia, in 46 cases, loss of two blood cell types (bilineage) with surviving red cells in a further 14 cases.

Virological examination of tissue samples using a range of techniques including cell culture, RT-PCR, electron microscopy and microarray analysis has found no evidence of any viral involvement in the syndrome.

Deep sequencing analysis of bone marrow looking for foreign nucleic acid from two affected calves has found no evidence of an infectious agent.

As the number of calves presenting with clinical disease is small in affected herds, the possibility of a genetic predisposition leading to disease was investigated. A small cohort of BNP cases was examined but there appeared to be no striking difference between the frequencies of the most common alleles in a case versus control comparison. The hypothesis that a limited number of alleles may be associated with disease does not appear to be the case.
SCI\textit{ENTIC OBJECTIVES}

1. To provide an accurate case definition of this new syndrome. This will be based on the results of the clinical examination of live, affected calves and the pathological examination of calves that have succumbed to disease. This objective will be accomplished after collaboration with colleagues from other European countries to ensure that we are all describing and investigating the same condition.

2. To identify, describe and characterise the case population. This will be achieved by identifying and describing temporal and geographic patterns of emergence of the condition. A survey of private veterinary surgeons (PVSs) from the VLA and SAC client-base will be conducted to gather this information and also to raise awareness amongst this sector of the veterinary profession.

3. To establish risk factors related to calves with IHD. A case-control study will be designed and 56 herds where clinical disease has occurred will be enrolled. Farmers will be interviewed using comprehensive and detailed questionnaires. The case population will be compared to a control population comprising 56 farms chosen at random.

4. The ultimate goal of this project, having defined a typical case, described the case population and risk factors related to its incidence, is to establish a definite cause. This will involve a detailed search for infectious agents, including novel viruses, and the possible role of colostrum uptake. In addition there will be a detailed analysis of all the epidemiological data to highlight other possible causes unrelated to exposure to an infectious agent.

5. To raise awareness of this new and emerging condition by communicating with, and transferring knowledge to, PVSs, farmers, the wider scientific community and risk managers. Results will be disseminated widely via the agricultural and scientific press and at meetings of both the scientific and lay community.

6. Finally, a vital objective is to evaluate the effectiveness of this approach to the investigation of a new syndrome of unknown cause that appears in our livestock population. This will encompass an evaluation of the efficiency of the investigation with respect to its timeliness, efficient use of resources, ability to generate meaningful data and the effectiveness of the collaborative effort of a number of organisations both within the UK and across other EU countries.

\textbf{Project Report to Defra}

8. As a guide this report should be no longer than 20 sides of A4. This report is to provide Defra with details of the outputs of the research project for internal purposes; to meet the terms of the contract; and to allow Defra to publish details of the outputs to meet Environmental Information Regulation or Freedom of Information obligations. This short report to Defra does not preclude contractors from also seeking to publish a full, formal scientific report/paper in an appropriate scientific or other journal/publication. Indeed, Defra actively encourages such publications as part of the contract terms. The report to Defra should include:

- the scientific objectives as set out in the contract;
- the extent to which the objectives set out in the contract have been met;
- details of methods used and the results obtained, including statistical analysis (if appropriate);
- a discussion of the results and their reliability;
- the main implications of the findings;
- possible future work; and
- any action resulting from the research (e.g. IP, Knowledge Transfer).
Extent objectives have been met

1. Accurate case definition
This objective was met, initially with a clinical definition and this was then superseded by a necropsy case definition incorporating the pathological finding of trilineage hypoplasia. This latter definition informed all our epidemiological studies and was in agreement with findings of European colleagues.

2. Characterise case population
   This objective was fully met in the case-series study conducted in 2009. See Appendix *

3. Establish risk factors
   This objective was fully met in the case-control study conducted in 2010. See Appendix **

4. Establish cause
   At the conclusion of the project a definitive cause has not been established. However despite exhaustive investigation no single specific aetiological agent has been found. Nonetheless a mechanism of disease has been proposed and a significant association with one specific vaccine product has been established. Suggestions for further studies to characterise the pathophysiological basis of this disease have been proposed elsewhere in this Report.

5. Raise awareness
   Extensive communication encompassing a wide range of interested parties has taken place throughout the duration of the project. See “Knowledge Transfer” section later in this report for full details.

6. Evaluate effectiveness of this approach to the investigation of a new syndrome

6 key strengths of the project were:

1. Work continued uninterrupted under the cattle scanning surveillance project whilst the contractual change to a research project was undertaken.

2. Collaboration with SAC allowed us to investigate on a GB basis – this has to be the right approach because GB is a single epidemiological unit with respect to new and emerging diseases.

3. Collaboration with MRI allowed us to integrate its areas of expertise.

4. During the course of the project useful collaboration with other EU partners took place both informally by e-mail and formally at scientific meetings.

5. The multi-disciplinary approach across the project was a particular strength. Characterisation of new diseases requires input from multiple disciplines – pathology, virology and epidemiology were key disciplines in this project. Pathology was crucial to inform the case definition and to help formulate hypotheses as to possible aetiologies and potential risk factors. Virology was crucial to address the likelihood of a novel viral disease which was of particular concern to risk managers because of the potential implications for public health. Epidemiology was crucial because of the strength of observational studies for exploration of potential risk factors, and for developing hypotheses on causation.

6. We managed to collaborate effectively with VMD.

With regard to timeliness all milestones were met except on one occasion when a scheduled meeting with Defra was postponed because of the unavailability of key staff due to other work commitments. The meeting was rescheduled at no detriment to the project.

Costs were almost kept within budget. The investigation of a new disease, by definition, is difficult to cost at the outset as the nature and direction of the investigation is not clearly defined. Meaningful data has hopefully been generated. An objective measure of this is acceptance of scientific papers by peer-reviewed scientific journals and I am optimistic that this will be achieved.

In conclusion I consider that robust good-value science has been delivered in a timely manner
Methods and Results

1. Case numbers and details

The first case of BNP was confirmed in Scotland on the 10/04/09, in England on 13/05/09 and in Wales on 09/10/09. These case details cover the period from 10/04/09 until 08/03/11. See Figure 1 for distribution of confirmed cases in GB.

Table 1. VLA cases and farms per Regional Laboratory (RL)

<table>
<thead>
<tr>
<th>VLA</th>
<th>No. of Cases Per RL</th>
<th>No. of Farms Per RL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bury</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Carmarthen</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Langford</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Leanhurst</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>Luddington</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Newcastle</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Penrith</td>
<td>15</td>
<td>46</td>
</tr>
<tr>
<td>Preston</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>RVC</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Shrewsbury</td>
<td>4</td>
<td>14</td>
</tr>
<tr>
<td>Starcross</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Sutton</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>Bonington</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thirsk</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>Truro</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Winch</td>
<td>3</td>
<td>8</td>
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</table>

* Farm also had case(s) in 2010
º Farm also had case in 2011

Table 2. Cases per month

<table>
<thead>
<tr>
<th>Year</th>
<th>No. of Cases Per Month (Total)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>2009</td>
</tr>
<tr>
<td>January</td>
<td>0</td>
</tr>
<tr>
<td>February</td>
<td>0</td>
</tr>
<tr>
<td>March</td>
<td>0</td>
</tr>
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<td>April</td>
<td>0</td>
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<tr>
<td>May</td>
<td>1</td>
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<tr>
<td>June</td>
<td>2</td>
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<tr>
<td>July</td>
<td>7</td>
</tr>
<tr>
<td>August*</td>
<td>9</td>
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<tr>
<td>September</td>
<td>18</td>
</tr>
<tr>
<td>October</td>
<td>9</td>
</tr>
<tr>
<td>November</td>
<td>5</td>
</tr>
<tr>
<td>December</td>
<td>3</td>
</tr>
</tbody>
</table>

* Free PMs ceased 07/08/10

Total Number of Dairy farms- 104
Total Number of Beef farms- 20
VLA BNP cases-Summary 13/05/09 to 08/03/11

Post mortem examination of calves from farms and histopathology of their bone marrow revealed trilineage hypoplasia consistent with a diagnosis of Bovine Neonatal Pancytopenia (see “Pathology” report: Section 4) on 124 farms. The calves were received from all Regional Laboratories except Aberystwyth. Many more cases were seen at the Penrith RL than at other RLs. Fewer cases were seen in Wales than would be predicted by livestock numbers in that country. Many more dairy calves than beef calves were seen by RLs in England and Wales. Total submissions received from 146 farms.

Calves

A total of 207 calves were received from these 146 farms

No evidence of BNP on bone marrow histopathology in 26 calves
BNP confirmed by bone marrow histopathology in 181 calves - 25 beef calves and 156 dairy calves

BNP confirmed by bone marrow histopathology-124 farms

**Beef**
- 3 beef farms confirmed in 2009
- 16 beef farms confirmed in 2010
- 1 beef farm confirmed in 2009 and 2010

**Dairy**
- 26 dairy farms confirmed in 2009
- 65 dairy farms confirmed in 2010
- 10 dairy farms confirmed in 2009 and 2011
- 1 dairy farm confirmed in 2009 and 2011
- 2 dairy farms confirmed in 2011

SAC BNP Submissions – Summary 10/4/09 to 28/2/11

Post mortem examination of 200 calves from 148 farms and histopathology of their bone marrow revealed trilineage hypoplasia consistent with a diagnosis of Bovine Neonatal Pancytopenia on 139 farms. Of these 139 farms 10 were beef farms on which BNP was diagnosed in 2009 and 2010, 1 was a dairy farm on which BNP was diagnosed in 2009 and 2010, 102 were beef farms with cases diagnosed in a single year only and 26 were dairy farms with cases diagnosed in a single year only. The calves were received by all 8 Disease Surveillance Centres in Scotland but were concentrated in the south east. Submissions received from a total of 148 farms.

Calves

A total of 200 calves were received from these 148 farms

No bone marrow histopathology carried out in 8
No evidence of BNP on bone marrow histopathology in 26
BNP confirmed by bone marrow histopathology in 166 calves - 135 beef calves and 31 dairy calves

BNP confirmed by bone marrow histopathology - 139 farms.

**Beef**
- 10 beef farms – BNP confirmed in 2009 and 2010
- 102 beef farms – BNP confirmed in a single year only (2009, 2010 or 2011)

**Dairy**
- 1 dairy farm - BNP confirmed in 2009 and 2010
- 26 dairy farms – BNP confirmed in a single year only (2009, 2010 or 2011)
Table 3. SAC cases and farms per Disease Surveillance Centre (DSC)

<table>
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<th>SAC</th>
<th>No. of Cases Per DSC</th>
<th>No. of Farms Per DSC</th>
</tr>
</thead>
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<tr>
<td>Aberdeen</td>
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<td>18</td>
</tr>
<tr>
<td>Ayr</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>Dumfries</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td>Edinburgh</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>Inverness</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Perth</td>
<td>5</td>
<td>14</td>
</tr>
<tr>
<td>St Boswells</td>
<td>10</td>
<td>45</td>
</tr>
<tr>
<td>Thurso</td>
<td>1</td>
<td>11</td>
</tr>
</tbody>
</table>

*Farm also had case(s) in 2010
°Farm also had case in 2011

Table 4. Cases per month

<table>
<thead>
<tr>
<th></th>
<th>No. of Cases Per Month (Total)</th>
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<tbody>
<tr>
<td></td>
<td>2009</td>
</tr>
<tr>
<td>January</td>
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<tr>
<td>February</td>
<td>0</td>
</tr>
<tr>
<td>March</td>
<td>0</td>
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<tr>
<td>April¹</td>
<td>7</td>
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<tr>
<td>May</td>
<td>7</td>
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<tr>
<td>June</td>
<td>3</td>
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<tr>
<td>July</td>
<td>5</td>
</tr>
<tr>
<td>August²</td>
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<tr>
<td>September</td>
<td>6</td>
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<tr>
<td>October</td>
<td>5</td>
</tr>
<tr>
<td>November</td>
<td>3</td>
</tr>
<tr>
<td>December</td>
<td>1</td>
</tr>
</tbody>
</table>

¹ 01/04/10 - Free PMEs ceased on second and subsequent submissions from farms where a case had already been confirmed in 2010.
² 01/08/10 – Free PMEs ceased

Therefore the above figures include those cases where only a gross diagnosis was made and no histopathology was carried out on the bone marrow.

Total Number of Dairy Farms – 28
Total Number of Dairy Calves – 34
Total Number of Beef Farms – 114
Total Number of Beef Calves – 141
Figure 1 Distribution of farms where BNP was confirmed between May 2009 and February 2011
2. Epidemiology

(a) Case-Series study 2009

Executive summary

- This report explores and describes 75 cases of bovine neonatal pancytopenia (BNP) in calves in Great Britain (GB), which were reported to the VLA and SAC from 24 April 2009 to the end of September 2009.

- The condition BNP appears to have emerged slowly through 2007 and 2008 and increased in frequency in 2009, but the small number of calf deaths attributed to this condition has as yet been an undetectable contribution to the overall mortality among young calves in the national herd.

- BNP calves were classified by histopathology into three different groups A (59 calves), B (2 calves) and C (1 calf). A total of 13 calves were not examined by histopathology and were classified similar to other confirmed cases on the farm.

- The clinical signs of BNP appeared on average around day 12 of age, but range from day 0 to day 20. The majority of calves die within a few days of showing clinical signs. Calves with a histopathology classification of B or C were more likely to die and show clinical signs at a very young age (mean=4), whereas calves with histopathology classification A showed clinical signs around day 13 of age. No calves classified in group A were reported to show clinical signs before day 2 of age.

- The majority of BNP calves received colostrum from their own dam.

- The majority of dams were vaccinated against Bluetongue and BVD during the pregnancy with the affected calf. Approximately two thirds of dams were vaccinated against Leptospira hardjo during this period. Because of the high immunisation load, it was not possible to separate the effect of the different immunisations.

- Farmer recall of brand of vaccines used may have been subject to inaccuracy and no conclusion could be made on whether a specific brand was implicated in the aetiology of BNP. The most popular brands were Bovilis BTV8 (Intervet/Schering-Plough Animal Health), PregSure BVD (Pfizer Ltd.) and Leptavoid H (Intervet/Schering-Plough Animal Health).

- The herds of origin of the cases were larger (mean=386) than the national average (mean=112). It is unknown whether this is a characteristic of the disease, associated with the cause or a characteristic of the VLA/SAC client base.

- Dairy calves were considered to be over-represented in the case population compared to the national distribution of purpose of herds. Organic herds were also considered to be over-represented.

- Further studies are needed to identify the specific aetiology of BNP.

A total of 75 cases with available husbandry information were enrolled between 24/04/2009 and 01/11/2009 (fig 1).

A summary of the main points of the case-series study are given below.
Figures 1 and 2 show the number of cases enrolled per week as part of the study and their geographical distribution in GB.

**Figure 1.** Number of cases enrolled per week between 24/04/09 and 01/11/2009. The 75 cases originated from 49 herds on 48 farms. On one farm, two calves originated from a dairy herd and a third from a separately managed beef herd. The majority of farms (88%) only had one or two calf cases investigated for this report, but three farms had 3 cases investigated and another three had 4 cases investigated. The average number of cases investigated per farm was 1.5 (median=1) and the maximum number of cases investigated per farm for this report was 4.

**Figure 2.** Geographical distribution of BNP cases in GB in 2009 and overall density of calves less than one month of age per 100km². (increased density with increased red colour)
Factors for further investigation

The herds of origin of the cases were larger (mean=386) than the national average (mean=112). Both dairy calves and organic herds were considered to be over-represented compared to national proportions. Another nine factors were found to be relevant for the majority (>60%) of cases and all factors will be investigated in the future study (table 1). Further information on husbandry factor of calf and dam can be found in the epidemiology report in Annex 1.

Table 1. Factors of potential association with IHD ranked according to frequency of reporting.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Number of calves</th>
<th>% of all calves</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dam vaccinated</td>
<td>75</td>
<td>100</td>
</tr>
<tr>
<td>Calf received colostrum</td>
<td>74</td>
<td>99</td>
</tr>
<tr>
<td>Calf received colostrum from own dam</td>
<td>68</td>
<td>91</td>
</tr>
<tr>
<td>Dam vaccinated against BVD during pregnancy</td>
<td>65</td>
<td>87</td>
</tr>
<tr>
<td>Dam fed grass after calving</td>
<td>59</td>
<td>86</td>
</tr>
<tr>
<td>Dam vaccinated against BTV8 during pregnancy</td>
<td>59</td>
<td>79</td>
</tr>
<tr>
<td>Dam fed grass 4 weeks before calving</td>
<td>55</td>
<td>79</td>
</tr>
<tr>
<td>Calf housed indoors</td>
<td>56</td>
<td>75</td>
</tr>
<tr>
<td>Buying in of cattle in last 12 months</td>
<td>50</td>
<td>67</td>
</tr>
<tr>
<td>Dam fed other feed after calving</td>
<td>45</td>
<td>65</td>
</tr>
<tr>
<td>Sheep present on farm</td>
<td>47</td>
<td>63</td>
</tr>
<tr>
<td>Dam vaccinated against Leptospira during pregnancy</td>
<td>46</td>
<td>61</td>
</tr>
</tbody>
</table>

Clinical signs

On average clinical signs appeared at the age of 12.5 days, ranging from 0 to 20 days of age and lasted on average 2.1 days ranging from 0 to 11 days. A variety of clinical signs were observed in case calves and the distribution is shown in table 2.

Table 2. Clinical signs reported for IHD calves via questionnaires ranked according to frequency.

<table>
<thead>
<tr>
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<tr>
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</table>

Two independent factors were identified and combined they explained the majority (82.8%) of the variance in clinical signs (fig 3). Factor 1 appeared to include all reported clinical signs, but the difference between the two factors was that factor 2 did not include the systemic signs such as malaise, recumbency and pyrexia. Diarrhoea and blood in faeces also scored low in factor 2. This suggested that the majority of calves showed both bleeding and systemic clinical signs, but some calves only showed bleeding without any of the systemic clinical signs. Factor 1 was not associated with histopathology classification, but calves classified as B or C were more likely to score highly in factor 2 i.e. less likely to show systemic clinical signs such as malaise, recumbency and pyrexia.
Figure 3. Loading plot illustrating the score of each sign within factor 1 and factor 2 that explains 83% of variation between clinical manifestations in IHD calves.

Information on both birth and death date was available for 65 of 75 calves. Of the remaining calves, 5 were reported to have survived. The distribution of age at time of death is shown in figure 4.

Figure 4. The age at time of death of 64 calves that died from IHD according to histopathology classification

(b) Case-Control study 2010

A full description of this study is to be found in a scientific paper by Lambton et al which was submitted for publication in a peer-reviewed journal on 10 May 2011. This scientific paper is entitled “Factors associated with bovine neonatal pancytopenia (BNP) in calves: a case-control study”. A summary of the paper and its abstract are presented below.

Executive summary

1. Relatively few herds have reported this condition
2. The disease has a high fatality rate.
3. Calves were ineligible for the study if they were no longer in their birth herd.
4. A case was defined as a calf younger than 29 days old, with unexplained haemorrhages detected at necropsy and trilineage hypoplasia of bone marrow confirmed by histopathology (see “Pathology” report, Section 4)
5. For each case calf, a control calf, matched by purpose of herd of origin, was retrospectively selected at random from the GB calf population.
6. Control calves were enrolled from herds with no history of BNP.

7. To be eligible to provide a control calf, a herd was required to have at least 100 adult female cattle and no history of BNP.

8. A herd was ineligible as a control herd if, from 01/01/09 to the end of the study period (31/08/09) any healthy male calves in the herd were humanely slaughtered or any calves (male or female) were sold before they reached 29 days old.

9. Control herds were monitored fortnightly by telephone enquiry to ensure that they continued to meet eligibility criteria.

10. If a calf in a control herd was diagnosed with BNP during the study period the herd was rejected and replaced.

11. Interviews were conducted with case and control herd owners using a standard questionnaire (see Appendix 2F)

12. Medicines Record Books were scrutinised at the time of the interview to validate medicines data.

13. Where dam vaccine records were not corroborated by an on-farm written or electronic record, farmers’ PVSs were contacted for relevant vaccine sales records.

14. All variables significant in univariate analyses were entered into a multivariable logistic regression model.

15. Of 58 control calves 24 were in herds in Scotland and 34 in herds in England; 49 were in beef suckler herds 9 in dairy herds

16. Multivariable logistic regression showed increased odds of a calf being a BNP case if:
   (a) The dam had received PregSure BVDv vaccine (OR 40.78)
   (b) The herd was located in Scotland (OR 9.71)
   (c) There was contact between the herd and sheep on the same premises (OR 14.6)

17. Decreased odds of a calf being a BNP case were associated with:
   (a) The calf having been kept outside (OR 0.11)
   (b) An increase in the number of years for which the herd had been established (OR 0.97)

18. The association with PregSure BVDV vaccine is strong and is independent of vaccine batch or dose effect.

Abstract

Bovine neonatal pancytopenia (BNP; previously known as idiopathic haemorrhagic diathesis and commonly known as bleeding calf syndrome) is a novel haemorrhagic disease syndrome of young calves which has emerged in a number of European countries during recent years. Data were retrospectively collected during June to September 2010 for 56 case calves diagnosed with clinical BNP between 17 March and 7 June of the same year. These were compared with 58 control calves randomly recruited from herds with no history of BNP. Multivariable logistic regression analysis showed that increased odds of a calf being a BNP case were associated with its dam having received PregSure BVD® (Pfizer Animal Health) vaccination prior to the birth of the calf (odds ratio (OR) 40.78, p < 0.001) and its herd of origin being located in Scotland (OR 9.71, p = 0.006). Decreased odds of a calf being a BNP case were associated with the calf having been kept outside (OR 0.11, p = 0.006) and the greater the length of time that the cattle herd had been established on the farm (OR 0.97, p = 0.011). There was also some evidence that contact between the herd and sheep on the same farm was associated with increased odds of a calf being a BNP case (OR 14.34, p = 0.005).

3. Virology

Virology performed at Central Veterinary Laboratory, Weybridge
Microarray:

Samples were tested by Microarray using Biochip v4.1 (www.bio-chip.co.uk). This version has about 9K oligonucleotides representing 29 virus families of veterinary/plant pathogens.

4 cases were tested by microarray for RNA viruses. Bovine coronavirus was detected in the intestinal contents on one calf, confirmed by PCR. This calf was shown to be suffering from a secondary coronavirus enteritis.

3 cases were tested by microarray for DNA viruses, with negative results in each case. In addition 6 cases were tested for circoviruses by RT-PCR, following a suggestion from a German worker that a virus similar to porcine circovirus-2 (PCV-2) had been detected in some of their samples. All tests proved negative. In addition samples were tested at Moredun using a pan-circovirus PCR (see Appendix 2- Table 4). A few samples have also been tested for torovirus and flavivirus by PCR, again with negative results.

Deep sequencing:

In collaboration with Liverpool University the bone marrow from 2 selected cases were subject to high throughput(deep) sequencing, a technique which, although less sensitive than primer-specific PCR, is capable of detecting the presence of any foreign DNA. This technique was adopted to either rule-in or rule-out the involvement of an infectious agent in this syndrome. It is ideal for full genome sequencing (e.g. viruses or bacteria) due to its ultra-high throughput and long single reads (500 base pairs). The two calves selected both showed characteristic bone marrow pathology and were submitted live to the RLs so their tissues were very fresh with minimal bacterial contamination.

RNA was isolated from the bone marrow of each calf. One sample each was analysed in Liverpool and Weybridge. 3300 and 13k sequences were obtained respectively. All sequences were mapped against the bovine genome and Ref Seq with 90% match expectancy. Numerous sequences were detected that did not map on first approach. This, however, may reflect the rather poor status of the bovine genome annotation. A series of ~250 sequences did not match the bovine genome at all and did not seem to reflect mammalian sequences at all. Those were blasted against GenBank and analysed individually. The majority resembled a crude mix of bacteria of all kind, both aerobic and anaerobic. Around five dozen sequences were completely unmatched by anything and were further investigated via their putative proteins.

Bone marrow from a suitably selected “control” calf was examined at the same time for evidence of similar unknown sequences, the assumption being that anything that occurs in both positive and negative samples can be disregarded, thus greatly simplifying the bioinformatic analysis. No differences were found and it was suggested that the unknown sequences may represent endogenous retroviral sequences of no significance.

Virology performed by the Virus Surveillance Unit of the Moredun Research Institute

103 samples from calves have been submitted; 49 from VLA centres and 54 from SAC centres or LAPTU. 6 placenta samples have also been submitted by LAPTU.

Virus isolation

Fifty four animals have had a variable number of tissues (2-7 per animal) tested by virus isolation in cell culture over 2-3 passes in BEK cells and by double monoclonal antibody ELISA (Entrican et al, 1995 Vet Micro, 43, 65) and real time RT-PCR for pestivirus detection and speciation (Willoughby et al 2006, J. Virol. Met. 2006, 137, 21). See Appendix 2-table 1 for detail of samples and tissues tested. All further samples received have been processed and stored for potential future testing.

Electron microscopy.

21 samples (from 12 animals) have been tested by electron microscopy by David Everest (VLA Weybridge), with negative results. (See Appendix 2-table 2). Passage numbers 1 and/or 3 were tested and samples were tested in both confluent and non-confluent cells, though not every sample was tested in both.

Other PCR testing

All testing was performed on RNA and DNA extracted from homogenised tissues rather than from material which has been through cell culture due to the possibility of detection of FCS contaminants.

Eight samples of bone marrow have been tested by pan pestivirus (324+326; Vilcek et al 1994, Arch Virol, 136, 309) and atypical (HoBi-like) pestivirus (Lui et al, 2008, J. Virol. Met. 154, 82) conventional RT-PCR and any amplicons detected, even of the wrong size, sequenced. No pestivirus or other virus sequence was detected (Appendix 2-table 3).
Nine bone marrow samples were tested with a pan-circovirus PCR (Todd et al, Avian Pathology 30 321; Johne et al JGV 87, 1189) amplicons cloned and sequenced; no circovirus DNA was detected but only one sample was cloned and studies are ongoing with this and a further PCR assay (Kappe, pers comm., Halami et al, Virus Res, 132, 208) (See Appendix 2-table 4).

9 samples (8 animals) from the outbreak on the farm attended by LAPTU, including three placenta samples, have been tested by real time RT-PCR for BRSV (Willoughby et al 2008, Vet Micro, 126, 264), and PI3 (Willoughby, unpublished) and pan-herspervirus PCR (Ehlers et al, 1999, Virus Genes, 18, 211) with negative results.( See- Appendix 2- table 5).
4. Pathology

VLA / SAC histopathology report

Summary

This report describes the histological findings in bone marrow of 354 calves examined as part of the investigations into a syndrome of unexplained haemorrhage in calves in Great Britain between 24.04.09 and 28.02.11.

346 calves met the case definition for inclusion in the study (less than 4 weeks of age, unexplained bleeding detected clinically or at necropsy, and no BVDV RNA detected). These 346 calves were grouped according to the histological changes in bone marrow. Group A calves (96%) had severe bone marrow injury, characterised by trilineage hypoplasia (‘aplastic anaemia’) involving marked depletion of erythroid and myeloid cells and megakaryocytes. Evidence of limited de novo haematopoietic cell regeneration in rib was detected in some group A calves. Group B calves (2%) had diffuse regenerative responses or no unequivocal change in bone marrow and Group C calves (1%) had combined depletion of both late stage erythroid series cells and megakaryocytes (bilineage hypoplasia).

The megakaryocyte loss (detected in calves in groups A and C) will have resulted in thrombocytopenia and hence abnormal bleeding. The mean calf age at the onset of clinical signs correlates fairly well with the expected interval between bone marrow injury and the onset of thrombocytopenia, thus would be consistent with a perinatal insult to the bone marrow. However some Group A calves developed clinical signs and died prior to 8 days of age, suggesting the possibilities of an additional peripheral mechanism of thrombocyte destruction and / or prenatal bone marrow injury, or of an additional process for example endotoxaemia associated with bacterial sepsis. There is no histological evidence of underlying primary immunodeficiency in calves with trilineage hypoplasia.

The lesions of trilineage hypoplasia are end-stage, and it was not possible to detect evidence of a causative agent at the time of examination. Reported causes of trilineage hypoplasia in cattle include bracken fern toxicity, toxicity associated with trichloroethylene extraction of soyabeans and possibly trichothecene mycotoxicosis. The clinicopathological findings militate against these.

In other species, recorded causes of or associations with trilineage hypoplasia include immune (T cell) mediated (this category accounts for many cases in man formerly classed as idiopathic), viral (parvovirus, retrovirus, herpesvirus, circovirus), medications and radiation. Recent communications suggest that BNP may represent an alloimmune pancytopenia (Bridger et al, 2011). This mechanism of trilineage hypoplasia does not appear to have been recorded in any species and the putative trigger for such a reaction is unknown.

Four < 4 weeks old calves with sporadic acute BVDV type 1-associated haemorrhage were also identified. This association between acute BVDV type 1 infection and a haemorrhagic diathesis in calves less than one month of age has not previously been recorded.

Veterinary investigation officers were requested also to submit samples from older cattle with unexpected bleeding for further investigation. Four such submissions were investigated, one with probable bracken toxicity, one with vasculopathy and bacterial hepatitis, one with probable disseminated intravascular coagulation of unknown cause and one with persistent pestivirus infection. None had lesions of trilineage hypoplasia in bone marrow. There is therefore no evidence to date that BNP is occurring in older cattle in GB.

Introduction

Following the identification of a novel bleeding syndrome of unknown cause in young calves in mainland Europe and subsequently in Scotland, a joint meeting of VLA and SAC veterinary investigation officers, pathologists, virologists and epidemiologists was held on 18-06-09 at the International Research Centre, Midlothian. At that meeting, clinical case criteria and a necropsy protocol were agreed for SAC and VLA investigations of this syndrome. The protocol included samples for virological, toxicological and histopathological analyses and was distributed to all SAC Disease Surveillance Centres and VLA Regional Laboratories. (See Appendix 4A)
Methodology and Results

Methodology

(1) VLA / SAC clinical case definition based on clinical/necropsy and virological criteria:

Clinical / necropsy:
- calves less than 4 weeks of age
- one or more of the following:
  - b. clinical features of unexplained / unexpected haemorrhage e.g. from injection or tagging sites, nares, rectum, skin
  - c. pancytopenia with thrombocytes < 20x10^9/L
  - d. necropsy findings of unexplained / unexpected haemorrhage at one or more sites

Virological:
- e. no BVDV RNA detected in spleen

(2) Histopathology and immunohistochemistry

The standardised necropsy sampling protocol included fixation of the following bone marrow samples in 10% neutral buffered formalin for histological evaluation: Femoral cavity, sternebrae 1 – 3 and distal ribs 6 - 8. The sternebrae and rib samples were decalcified for 6-8 hours in a ‘rapid decalcifying’ solution before routine processing to H&E sections. Selected sections of bone marrow and lymphoid tissues were immunostained for a range of CD markers and compared with those of unaffected calves in the same age range. In addition, bone marrow sections from randomly selected cases of BNP were immunostained for porcine circovirus 2.

(3) Archival studies

The histology archives of VLA were searched for submissions of bone marrow samples from calves less than 30 days between 1999 and 2008, and the histology of these cases reviewed against the results in BNP-affected calves.

(4) Terminology

Alloimmune: immune response characterised by production of antibodies reactive to alloantigens, that are recognised as different by different individuals of the same species.

Aplastic anaemia: a deficiency of the cellular elements of blood (specifically erythrocytes, leukocytes, and platelets), representing a failure of the haematopoietic cell-generating capacity of bone marrow (trilineage hypoplasia). The term aplastic anaemia is misleading as it involves more than just red blood cells. Diagnostic criteria for aplastic anaemia in man also include haematological criteria for pancytopenia as well as histological demonstration of trilineage hypoplasia in bone marrow.

Haematopoiesis: production of blood cells involving 3 main cell lineages:
- Erythroid series cells: produce red blood cells
- Myeloid series cells: produce granulocytes (neutrophils, eosinophils)
- Megakaryocytes: produce thrombocytes (platelets)

Hypoplasia: Atrophy due to destruction of some of the elements of a tissue or organ.

Pancytopenia: reduction in circulating granulocytes, thrombocytes and red blood cells.

Regeneration: the process of repair, reproduction, or replacement of lost or injured cells, tissues, or organs.

Trilineage hypoplasia (TLH): Concurrent depletion of erythroid and myeloid cells and megakaryocytes from bone marrow. Trilineage hypoplasia is the histological criterion for diagnosis of aplastic anaemia. Diagnostic criteria for aplastic anaemia in man also include haematological criteria for pancytopenia as well as histological demonstration of trilineage hypoplasia in bone marrow.

Results
(1) Numbers of calves examined histologically at 28.02.11
Samples of bone marrow from 354 calves have been examined histologically.

(2) Calves meeting the VLA / SAC clinical case definition for BNP
346 calves fully met the VLA / SAC case definition and were examined. Histological analyses of bone marrow of these calves identified the following 3 groups.

Group A: Marked trilineage hypoplasia (aplastic anaemia) involving extensive depletion of erythroid and myeloid precursors and megakaryocytes was observed in 333 (96.2 %) calves. In some cases small and occasional larger foci of haematopoesis were also present.
Group B: Diffuse regenerative responses or no unequivocal change in eight calves (2.4%).
Group C: Bilineage hypoplasia in four calves (1.2%) with combined marked depletion of both late stage erythroid series cells and megakaryocytes.
One calf had bone marrow lesions that were intermediate between group A and group C.

(3) Calves included in the case-series study at 30.09.09
62 calves both met the case definition for inclusion in the study and had calf investigation questionnaires completed for epidemiological analyses. These were categorised as trilineage hypoplasia (59), bilineage hypoplasia (1) and regenerative response (2).

(4) Calves excluded from the study
No evidence that idiopathic trilineage hypoplasia is occurring in cattle older than 30 days.

(5) Evaluation of bone marrow from sternum and rib
These observations suggest a de novo regenerative response.

(6) Assessment of optimum bone marrow sampling sites for confirmation of bone marrow lesions of bovine neonatal pancytopenia
Sternum is the optimum sample for detection of histological lesions of trilineage hypoplasia. Histological evaluation of sternum provides a suitable means for screening for evidence of trilineage hypoplasia in dead calves from control herds in the Case control study

(7) Assessment of lymphoid tissues and immunohistochemistry
No histological evidence of lymphoid hypoplasia was detected in calves with BNP compared with unaffected calves in the same age range. The thymic atrophy observed in both groups of calves is likely to be secondary. The presence of macrophage and CD3+ cell populations in bone marrow of calves with TLH raises the possibility of an immune-mediated pathogenesis. Further detailed work is required to investigate this possibility.

(8) Archival studies
The histology archives of VLA and SAC were searched for submissions of bone marrow samples from calves less than 30 days between 1999 and 2008. Two calves with abnormal bleeding submitted for investigation in 2008 had lesions of trilineage hypoplasia in bone marrow.

COMMENTS
The large majority of calves that met the VLA/SAC clinical case definition [based on clinical/necropsy and virological criteria] had lesions of trilineage hypoplasia in bone marrow. Trilineage hypoplasia is the histological criterion for the diagnosis of aplastic anaemia in man.

The presence of trilineage hypoplasia indicates that the pathogenesis includes injury to pluripotential stem cells in bone marrow. In group A and C calves, the marked depletion of megakaryocytes would account for the observations of thrombocytopenia and abnormal bleeding. Thrombocytopenia is expected to develop about 8 – 10 days after bone marrow injury (Weiss, 2006). This time interval correlates fairly well with the onset of reported clinical signs at day 12 (mean) which would therefore be consistent with a perinatal insult to bone marrow stem cells. However some Group A calves developed clinical signs and died prior to 8 days of age, suggesting the possibilities of an associated peripheral mechanism of thrombocyte destruction and / or prenatal bone marrow injury, or of an additional process for example due to endotoxaemia associated with bacterial infections.
The lesions of trilineage hypoplasia present in the group A calves are end-stage, and it was not possible to detect evidence of a causative agent at the time of examination.

Reported causes of trilineage hypoplasia / aplastic anaemia in cattle include bracken fern toxicity, soybean meal extracted with trichloroethylene resulting in DCVC (S-1,2 dichlorovinyl-L-cysteine) toxicity, furazolidone intoxication and possibly trichothecene mycotoxicosis. The clinicopathological findings including the presence of a de novo regenerative response in some calves strongly militate against these causes. Single cases of pancytopenia associated with bone marrow aplasia have been reported in calves in Canada and Japan for which a possible cause was not found (Ammann et al 1996; Shimada et al 2007).

In other species, recorded causes of or associations with aplastic anaemia include immune (T cell) mediated (this category accounts for many cases in man formerly classed as idiopathic), viral (parvovirus, retrovirus, herpesvirus, circovirus), medications (eg oestrogens, chemotherapeutic agents, NSAIDs, antibiotics) and radiation. The clinical and histological findings in group A calves bear some resemblance to those associated with vertical transmission of chicken anemia virus (CAV; avian circovirus). The clinical signs of haemorrhage and pallor associated with CAV begin about 10-12 days of age with a peak at 17-24 days; histologically there is trilineage hypoplasia (aplastic anaemia) with haematopoietic cells being replaced by adipocytes and stromal cells and regenerative foci of blast cells appear 16 – 18 days after experimental infection. However, virological and immunohistochemical investigations found no evidence of circovirus infection in cases of BNP in GB.

Recent communications suggest that BNP may represent an alloimmune pancytopenia following colostral intake (Friedrich et al 2011; Bridger et al 2011). This mechanism of trilineage hypoplasia does not appear to have been recorded in any species and the putative trigger for such a reaction is therefore unknown. Alloimmune anaemia and thrombocytopenia etc are well recognised in other species particularly in man, however a recent review of aplastic anaemia in childhood, (Guinan, 2009) does not record an alloimmune mechanism as a possible cause.

It is notable that 2 of the 4 calves with bilineage hypoplasia (megakaryocyte/erythroid) originate from herds in which at least one other calf in the herd had confirmed cases of BNP with lesions of trilineage hypoplasia, and it is therefore possible that megakaryocyte / erythroid bilineage hypoplasia represents a variant of BNP, although further work is required to clarify this. A variation in targets of alloimmune antibodies to include peripheral and / or stem cells might explain these differing presentations. It is likely that the group B calves without haematopoietic cell depletion represent different aetiopathogenetic entities.

Two < 4 weeks old calves with sporadic acute BVDV type 1-associated thrombocytopenia and haemorrhage were also identified. This association between BVDV type 1 and a haemorrhagic diathesis in young calves has not previously been recorded.

VIOs were requested to submit samples from older cattle with unexpected bleeding for further investigation. Three such submissions were investigated, one with probable bracken toxicity, one with vasculopathy and bacterial hepatitis and one with persistent pestivirus infection. There is therefore no evidence to date that BNP is occurring in older calves in GB.

REFERENCES


Friedrich, A., Buttner, M. and others (2011) Ingestion of colostrum from specific cows induces Bovine Neonatal Pancytopenia (BNP) in some calves. BMC Veterinary Research, 7, 10


5. Immunology

Do cattle that deliver affected calves have serum antibodies that react with molecule(s) expressed on the surface of neonatal bone marrow cell population(s)? To investigate this, serum samples from affected and unaffected herds, and viable neonatal bone marrow, were supplied by the Large Animal Practice Teaching Unit at the R(D)VS. Bone marrow was analysed by FACS to determine whether antibody in the serum from dams of affected calves binds to neonatal bone marrow. If such an effect is detected, future experiments will be designed to investigate cell lysis.

Serum samples from a herd that produced affected calves as well as from a second herd (unvaccinated, BVDV negative) was collected and stored at -70°C. In the affected herd, 5 animals had previously confirmed cases of BNP, 5 delivered normal calves and 9 had calves with sub-clinical presentations. Aliquots of serum were heat inactivated before use to prevent complement-mediated lysis of cells.

A pilot study was initiated to investigate the optimal conditions to harvest and process neonatal bone marrow. Bone marrow was collected from newborn calves immediately after euthanasia and processed either fresh or cryopreserved bone marrow for flow cytometric analysis. The major difference observed was that cryopreserved bone marrow lost red blood cell contaminants and had an increased number of dead cells after resuscitation. Cryopreserved bone marrow was initially tested with a 1/20 and a 1/100 dilution of dams sera. RBC-lysed whole blood from unaffected adult cattle was also included as positive control for the flow cytometric staining. Primary antibody binding was detected with rabbit anti-bovine IgG, followed by anti rabbit-Alexa 488 conjugate. Cells were analysed by flow cytometry, acquiring a minimum of 10,000 cells/sample. Antibody binding to cells was evaluated as shift (increase) of the level of 530±40 nm fluorescence of the cellular population.

There was no binding of dam’s sera to the cryopreserved bone marrow (no shift in fluorescence detected) with any of the sera investigated, from either herd. However, the positive control (adult blood cells), showed a shift in the fluorescence pattern associated with 4 out of the 5 animals that had produced affected animals, whereas the results obtained with the sera from the negative herd were consistently negative (see graphs below).

**Left**, histogram of fluorescence of PBMCs tested with different dam’s sera from the affected herd and analysed by flow cytometry. Horizontal axes represent intensity of fluorescence whereas vertical axis represent number of cells. Red: negative control (autologous sera); green affected; blue affected; orange normal; light blue affected; pink affected, black affected.

**Right**, histogram of fluorescence of PBMCs tested with different dams sera from the affected herd and analysed by flow cytometry. Red: negative control (autologous sera); all other colours: negative animals.

Freshly collected and stained bone marrow was also tested for antibody binding but did not show any shift in fluorescence. A cytospin analysis of the bone marrow preparation showed that it lacked megakaryocytes which are thought to be a major target population during the disease, possibly explaining the lack of binding. In addition, it is not possible to exclude that other cellular subpopulations have been lost during either the processing or the acquisition stage of the FACS analysis.

Future work will follow up the binding of sera to normal blood by quantifying and standardizing the amount of IgG present in the sera, to ascertain if the binding detected is due to a higher IgG content or if is animal-specific. Currently, the plans for testing cell lysis following antibody binding have been put on hold until an appropriate cell target is identified.
6. Genetics

A preliminary analysis of MHC genetic diversity and susceptibility to Bovine Neonatal Pancytopenia (bleeding calf syndrome)

Introduction
Bovine Neonatal Pancytopenia (bleeding calf syndrome) is a recently described disease of unknown aetiology. This disease of new-born calves has been described in a number of North European countries over the last 18 months and it appears to be associated with an inappropriate maternal antibody mediated immune response. As the number of calves presenting with clinical disease is small in affected herds, there may be a genetic predisposition leading to disease. The Major Histocompatibility Complex (MHC) includes polymorphic loci directly involved in the induction of antibody mediated immune responses. Many associations have been described between MHC class II allelic diversity and susceptibility of autoimmune and infectious diseases. We therefore sought to genotype affected calves at the MHC class II DRB3 locus and to compare allelic frequencies with those previously defined in breed matched disease free animals. The study concentrated on only Holstein dairy cattle from multiple holdings in order to reduce the expected levels of allelic diversity and because data was available from previous analyses for comparison of allelic frequencies. No analysis of beef cattle cases was carried out.

Results
Twenty four genomic DNA samples were typed at the BoLA-DRB3 locus by direct sequence analysis of a PCR product including the polymorphic the second exon. Individual alleles were identified by searching a database of all known alleles. Table 1 shows the alleles identified in the cohort. The frequency of each allele is shown in Figure 1 and Table 2. Twelve alleles were identified in the 24 animals, however only 4 occurred more than twice in this cohort. The top three alleles, *0101, *1101 and *1001 occurred in 80% of animals in this cohort.

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<td>19</td>
<td>*1002</td>
<td>*1101</td>
</tr>
<tr>
<td>20</td>
<td>*1001</td>
<td>*2002</td>
</tr>
<tr>
<td>21</td>
<td>*0101</td>
<td>*1101</td>
</tr>
<tr>
<td>22</td>
<td>*0101</td>
<td>*1501</td>
</tr>
<tr>
<td>23</td>
<td>*0101</td>
<td>*0902</td>
</tr>
<tr>
<td>24</td>
<td>*1201</td>
<td>?</td>
</tr>
</tbody>
</table>

Table 2 BoLA-DRB3 Allelic frequencies in BNP calves
Comparison of allelic frequencies in BNP calves with previous data from 32 Holstein cattle. The allelic frequencies of BoLA-DRB3 alleles from 32 randomly selected Holstein cattle sampled between 2008 and 2009 are shown in Table 3.

Table 3 BoLA-DRB3 allele frequencies from 32 Holstein cattle from 4 different holdings
In this cohort of animals the three highest frequency alleles were identical to those in the BNP cohort suggesting no radical difference in the distribution of alleles between the two sample groups.

<table>
<thead>
<tr>
<th>Number</th>
<th>Frequency</th>
<th>Allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15.63</td>
<td>*1001</td>
</tr>
<tr>
<td>2</td>
<td>14.06</td>
<td>*0101</td>
</tr>
<tr>
<td>3</td>
<td>10.94</td>
<td>*1101</td>
</tr>
<tr>
<td>4</td>
<td>10.94</td>
<td>*1801</td>
</tr>
<tr>
<td>5</td>
<td>10.94</td>
<td>*2701</td>
</tr>
<tr>
<td>6</td>
<td>7.81</td>
<td>*1501</td>
</tr>
<tr>
<td>7</td>
<td>7.81</td>
<td>?</td>
</tr>
<tr>
<td>8</td>
<td>6.25</td>
<td>*3201</td>
</tr>
<tr>
<td>9</td>
<td>6.25</td>
<td>*1201</td>
</tr>
<tr>
<td>10</td>
<td>4.69</td>
<td>*0901</td>
</tr>
<tr>
<td>11</td>
<td>1.56</td>
<td>*0601</td>
</tr>
<tr>
<td>12</td>
<td>1.56</td>
<td>?</td>
</tr>
<tr>
<td>13</td>
<td>1.56</td>
<td>*1401</td>
</tr>
<tr>
<td>14</td>
<td>15.63</td>
<td>*1001</td>
</tr>
</tbody>
</table>

Conclusions
From this small cohort of BNP cases there appears to be no striking difference between the frequencies of the most common alleles in this case versus control comparison. Our original hypothesis that a limited number of alleles may be associated with disease does not appear to be the case.

7. Haematology
Haematology Results
Blood samples were taken from some live, suspect cases of BNP and these were all tested at SAC Edinburgh. Samples were collected using standard kits supplied by VLA/SAC, according to a prescribed protocol (available on request).

Codes used for haematological interpretation:

1 one cell line only affected
2 two cell lines affected (all but one in this group had granulocytes and platelets affected.
3 all three cell lines affected, pancytopenia
SR suspect case, but probably sampled while recovering

Marked neutropenia and thrombocytopenia together with a variable degree of anaemia, often profound, plus the clinical presentation in calves of 7 to 30 days old is highly suggestive of BNP and was seen in the majority of clinical cases. A number of cases had relatively spared erythrocyte lines, an inevitable finding in rapidly progressing cases because of the relatively long erythrocyte half-life

Neutropenia or thrombocytopenia, together with anaemia and the clinical presentation, is suspicious and was seen in a minority of cases.

A single cell line affected may represent a regenerative response in an affected calf or may not be a clinical case of BNP.

Haematological data obtained supported the observation that clinical cases do not present at under 7 days or older than 28 days of age.

Table 1: Results from blood samples taken during the study: VLA 2009

<table>
<thead>
<tr>
<th>Test result</th>
<th>Observed anomaly</th>
<th>Number of cases</th>
<th>Confirmed BNP</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>IHD cases</td>
<td>Pancytopenia</td>
<td>17</td>
<td>17</td>
<td>3 cell lines affected</td>
</tr>
<tr>
<td>Probable</td>
<td>Pancytopenia but no</td>
<td>2</td>
<td>2</td>
<td>May be early cases or sampled during recovery or different form of BNP</td>
</tr>
<tr>
<td>cases</td>
<td>anemia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Possible</td>
<td>Only one cell line</td>
<td>3</td>
<td>0</td>
<td>Unusual or non-cases</td>
</tr>
<tr>
<td>cases</td>
<td>affected</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Results from blood samples taken during the study: SAC 2009

<table>
<thead>
<tr>
<th>Test result</th>
<th>Observed anomaly</th>
<th>Number of cases</th>
<th>Confirmed IHD by</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>IHD cases</td>
<td>Pancytopenia</td>
<td>4</td>
<td>4</td>
<td>3 cell lines affected</td>
</tr>
<tr>
<td>Probable</td>
<td>Pancytopenia but no</td>
<td>4</td>
<td>4</td>
<td>May be early cases or sampled during recovery or different form of BNP</td>
</tr>
<tr>
<td>cases</td>
<td>anemia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unlikely</td>
<td>Only one cell line</td>
<td>0</td>
<td>0</td>
<td>Unusual or non-cases</td>
</tr>
<tr>
<td>cases</td>
<td>affected</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Results from blood samples taken during the study: VLA 2010

<table>
<thead>
<tr>
<th>Test result</th>
<th>Observed anomaly</th>
<th>Number of cases</th>
<th>Confirmed IHD by</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>IHD cases</td>
<td>Pancytopenia</td>
<td>7</td>
<td>7</td>
<td>3 cell lines affected</td>
</tr>
<tr>
<td>Probable</td>
<td>Pancytopenia but no</td>
<td>1</td>
<td>1</td>
<td>May be early cases or sampled during recovery or different form of BNP</td>
</tr>
<tr>
<td>cases</td>
<td>anemia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unlikely</td>
<td>Only one cell line</td>
<td>0</td>
<td>0</td>
<td>Unusual or non-cases</td>
</tr>
<tr>
<td>cases</td>
<td>affected</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4: Results from blood samples taken during the study: SAC 2010
Full results for samples tested in 2009 and 2010 can be found in Appendix 5A

Haematological Studies on a Case Farm in Scotland

(joint funded by SAC/DEFRA and the RCVS trust)

BACKGROUND
The Farm Animal Practice at the Royal (Dick) School of Veterinary Studies (University of Edinburgh) has access to a farm that had 10 cases of Bovine Neonatal Pancytopenia (BNP) in 2009. It was reported at the European Buiatrics Congress (Friedrich et al, 2009a and Pardon et al, 2009) that dams of BNP calves are likely to produce BNP calves in subsequent years. The Farm Animal Practice client was therefore considered likely to have further cases of BNP in 2010 and was recruited as a study farm for investigations into this novel condition.

GENERAL REPORT ON THE STUDY FARM
200 cow beef suckler unit in East Lothian
Farm had 10 cases of BNP in Spring 2009
Mixed cross-bred cows bred to Angus, Charolais and Simmental bulls
Herd vaccinated against BVDV (Pregsure; Pfizer), Leptospirosis hardjo (Leptavoid H; Schering Plough), and Rotavirus, Coronavirus, and Escherichia coli K99 (Trivacton 6; Merial) with booster vaccination given during pregnancy
Additionally vaccinated with Bluetongue virus serotype 8 (Zulvac 8 Bovis; Forte Dodge) in 2009 but not 2010
Cows housed over winter and during calving, and fed a mixture of grass silage, cereal by-products, liquid urea protein and proprietary minerals with access to straw bedding.
Calving is now complete for Spring 2010
Cows appeared healthy and calves were a good birth weight
Abortion rate (2009 and 2010) was <3%
Neonatal mortality rate, excluding BNP cases, (2009 and 2010) was 2% from birth to >6wks old

BOVINE NEONATAL HAEMATOLOGY
Bovine neonatal haematology differs significantly from the adult bovine and changes significantly with the age of the calf
Only limited data is available to provide reference ranges – see Appendix 5B for ranges used in this study
It is hoped that when analysis of the data in this study is complete it will provide additional reference data
All samples were taken with needle and syringe from the jugular vein into an EDTA tube (for haematology) and submitted to the lab within 6 hrs of collection for the preparation of a fresh blood smear

CLINICAL CASES OF BNP
4 fatal cases of BNP confirmed by bone marrow histopathology
The dam of one calf was a heifer, while the other 3 dams were cows that reared apparently healthy calves in 2009
BNP calves sired by 3 different bulls

Note – the 10 dams of the 2009 BNP cases were enrolled on a BNP prevention trial (see aim 3) and so are not included in 2010 case figures

Haematology
All had normal haematology when sampled at <24 hours old, (with the exception of a low lymphocyte count in one calf.)
Mean platelet count = 351 x109/L (range 270-410 x109/L)
Mean neutrophil count = 8.8 x10⁹/L (range 4.1-12.1 x10⁹/L)
Mean lymphocyte count = 2.5 x10⁹/L (range 0.1-5.0 x10⁹/L)
Mean erythrocyte count = 9.4 x10¹²/L (range 8.1-10.0 x10¹²/L)
At the onset of clinical signs all 4 calves had a profound thrombocytopenia and leukopenia with a mild anaemia.
At the time of last sampling before death/euthanasia all calves had a profound hypoplastic pancytopenia
Mean platelet count = 13 x10⁹/L (range 0-25 x10⁹/L)
Mean neutrophil count = 0.03 x10⁹/L (0-0.1 range x10⁹/L)
Mean lymphocyte count = 0.9 x10⁹/L (0.4-2 range x10⁹/L)
Mean erythrocyte count = 4.9 x10¹²/L (1.9-6.7 range x10¹²/L)

Further analysis of this haematology is required but it seems to support the suggestion that BNP may be caused by a colostrum derived factor, with haematology being normal at birth and subsequently deteriorating in accordance with the lifespan of peripheral blood cell.

Clinical Signs
Clinical signs started at 16, 12, 19 and 14 days (mean = 15 days)
One calf showed no signs of external bleeding but presented with dyspnoea and lethargy which progressed to recumbency, with necropsy revealing marked haemorrhage into the lung parenchyma
Signs of bleeding in the other 3 cases were subtle; mild crusty epistaxis, dried blood around ear tags and petechial haemorrhages on the gums
All calves were pyrexic at the onset of clinical signs and remained pyrexic until the terminal stages of the disease
For full details of study methods see Appendix 5B.

AIM 1 (INVESTIGATING THE DAMS OF BNP CALVES)
Background
Very little investigation has been done into the dams of calves affected with BNP, although there is limited data that suggests that dams of BNP calves show haematological abnormalities (Bell et al 2009a, Muller et al 2009). This was investigated by sampling cows on an affected farm during pregnancy.

Aims
To determine if there are any haematological abnormalities in cows on a single farm affected with BNP, specifically;
- Any differences between BVDV vaccinated and unvaccinated adult bovines
- Any differences between dams of BNP calves and dams of haematologically normal calves
- Any differences during pregnancy compared to after calving in dams of BNP calves

Preliminary Results / Progress
All haematology was with the normal range for adult bovines with no differences between any of the groups

AIM 2 (INVESTIGATING SUBCLINICAL FORMS OF BNP)

Background
Subclinical forms of BNP have been described (Bell et al 2009a, Pardon et al 2009) where apparently healthy calves have been shown to have haematological abnormalities. However data so far has been limited and the incidence of subclinical disease on affected farms is unknown.

Aims
To determine the incidence of subclinical BNP on one affected farm
To correlate haematological abnormalities with colostral Ig levels
To accurately ascertain the temporal pattern of BNP
To determine if there are any haematological abnormalities in calves on an affected farm prior to colostrum ingestion
To collect samples from the population of calves on an affected farm for potential future genetic analysis

Preliminary Results / Progress
16 pre-colostral samples collected all showed normal haematology. Some abnormal haematology results suggestive of subclinical disease have been seen. 14 calves with no clinical signs of BNP showed a reduction in platelet count between birth and 10-14 days old. As the normal ranges for platelet counts in neonatal calves have not been established the significance of this is unknown, however ongoing research at the Moredun research Institute may shed more light on this. Early indications are that this suggests the presence of subclinical disease.

AIM 3 (PREVENTING BNP WITH COLOSTRUM SUBSTITUTION)

Background
Work in Germany (Friedrich et al, 2009b) has suggested that colostrum plays a role as an aetiological agent in Bovine Neonatal Pancytopenia (BNP). However this study was lacking control animals and gave no detailed information about cases occurring on the farm where the colostrum was collected.

Aims
To determine if preventing calves born to BNP affected cows from suckling their own dam’s colostrum prevents them from developing haematological changes and clinical signs consistent with BNP
To store colostrum from BNP affected cows for future work investigating the role of colostrum as an aetiological agent

Results
All calves have remained free of clinical signs of BNP
All are alive and healthy
PRELIMINARY CONCLUSIONS

Cases of BNP may present with no signs of external haemorrhage, or only very mild signs of external haemorrhage.

Haematology from BNP cases appears to have normal haematology at <24 hours old.

Haematology from BNP cases seems to support the suggestion that BNP may be caused by a colostrum derived factor, with haematology being normal at birth and subsequently deteriorating in accordance with the lifespan of peripheral blood cell.

Substituting colostrum for the first 32 hours of life allowed dams of calf that had died of BNP in 2009 to successfully rear a healthy calf in 2010.

REFERENCES


Discussion of results

1. Epidemiology
The case-series study in 2009 was able to identify factors for further investigation in the case-control study in 2010. The case-control study was used to identify risk factors and estimate the quantitative effects of the various exposures which may contribute to BNP. The use of this methodology, used with clearly defined inclusion and exclusion criteria, is a standard study design suited to the investigation of rare diseases. A case-control study is relatively quick to conduct and allows for the study of multiple exposures. However, a case-control study cannot prove causation or measure disease prevalence. The study resulted in the identification of a number of factors with a significant association with BNP, which included the use of the BVDv vaccine, PregSure BVD, in the affected calf's dam at any time in the last five years, if the calf was in a Scottish herd and if the calf had been housed. Risk of BNP was decreased in herds on premises which had farmed cattle for longer.

2. Virology
Using all currently available techniques no evidence of a viral or indeed any other infectious agent has been found. These findings are in line with those of other work groups across Europe. To ensure reliability and consistency the virological investigations were carried out in only two laboratories, one in Scotland and one in England. Results from each laboratory were in full accord.

3. Pathology
The samples for histology were collected according to an agreed necropsy protocol for suspect cases. Typical BNP calves had severe bone marrow injury, characterised by trilineage hypoplasia ('aplastic anaemia') involving marked depletion of erythroid and myeloid cells and megakaryocytes. The megakaryocyte loss will have resulted in thrombocytopenia and hence the abnormal bleeding seen externally and/or internally in all of the affected calves. Evidence of limited de novo haemoipoietic cell regeneration in rib was detected in some. It is concluded that histological examination of sternum is a robust means of confirmation of necropsy confirmation of BNP as the lesions are consistently present at this site and are sufficiently different from bone marrow pathology of other calfhood disease to be characteristic of BNP. Examination of this tissue in preference to other sites provided the cornerstone for the diagnosis and hence case definition of BNP. Histological evidence of lymphoid hypoplasia was not detected. The presence of CD3+ cell and macrophage populations in the bone marrow of affected calves suggests the possibility of an immune-mediated pathogenesis. Further work is required to investigate this possibility.

4. Haematology
A consistent haematological picture has been found in almost all those clinically affected calves sampled before death. Classically, all three bone marrow cell lines have been affected, red cells, white cells and platelets, hence the name "pancytopenia". Red cell half-life is much longer than neutrophil and platelet half-lives and therefore some cases have presented clinically before red cell numbers have fallen dramatically. Although these results are not pathognomonic for BNP they are highly suggestive when taken in association with clinical signs. To ensure reliability and consistency ALL haematological examinations were carried out in one laboratory in Scotland and samples were taken as per a defined protocol using needle and syringe with no use of a vacutainer.

5. Immunology
Bone marrow was analysed by FACS to determine whether antibody in the serum from dams of affected calves might bind to neonatal bone marrow but we were unable to demonstrate this effect. However this still remains the most plausible aetiological hypothesis and future work should continue to investigate the likelihood of this mechanism.

6. Genetics
This work was carried out in a single laboratory in Edinburgh using tried and reliable techniques. It was postulated that there may be a genetic predisposition leading to disease; however from a small cohort of BNP cases there appears to be no striking difference between the frequencies of the most common alleles in a case versus control comparison. Our original hypothesis that a limited number of alleles may be associated with
Main Implications of the findings

(1) The study has found no evidence of an infectious aetiology for BNP. The likelihood that this syndrome is a zoonosis is therefore considered remote.

(2) A significant association has been found between the use of PregSure BVDv vaccine in the dam and the occurrence of this syndrome in her calf. In light of this association, the threat of BNP to our national cattle herd has been mitigated by the withdrawal of this vaccine from the European market in 2010. Alternative vaccine products are available to control this disease in GB and no association has been found between these alternative products and the syndrome of BNP.

(3) Although our study has provided some information on the pathogenesis of BNP, the underlying mechanism initiating the disease process is still unclear. The exact role of the PregSure vaccine is uncertain and it must be borne in mind that, although observational epidemiological studies can reveal associations between certain exposures and a disease, they do not confirm causation. It is anticipated that with the removal of this product from the marketplace the incidence of this syndrome will steadily reduce over the next few years. Because of the gaps in our knowledge about the pathogenesis of BNP, it is not possible at this stage to anticipate the likelihood of such a syndrome or related syndromes appearing in the future in association with other vaccine products.

Possible Future Work

Investigations to date support an immune-mediated pathogenesis, most likely associated with the presence of alloantibody in maternal colostrum. An association with a particular brand of BVDv vaccine has been shown.

Hypotheses that we would wish to investigate, in possible future work, include the following:

(1) We hypothesise that BNP is an alloimmune disease associated with absorption of maternal colostral antibody reactive with a target expressed on neonatal bovine haemopoietic cells. Future work to identify potential target antigens is suggested.

(2) It is possible that use of the implicated vaccine is directly causal (for example, due to production of antibodies that are cross-reactive between a vaccine component, such as the cell line or serum products employed in production, and an antigen present on some neonatal calf haemopoietic cell lineages). Further in vitro studies of the binding properties of serum/colostral antibody of affected and control dams on neonatal bone marrow sections/cells should be performed to further elucidate this potential interaction. Access to the cell lines and virus strains used by the manufacturer would be helpful.

(3) It is also possible that use of the implicated vaccine increases production of alloantibodies already present at low (subclinical) levels in some animals thereby explaining the sudden increase in the prevalence of BNP since 2008. This may be due to either the biological agents used in vaccine production as at 2 above or due to another component of the vaccine, such as the adjuvant system employed, increasing alloantibody production in pregnancy. Further work to investigate the immunology of pregnancy in dams producing normal and affected animals may be informative, and studies on the effect of the vaccine and/or its component fractions could help elucidate the apparent immunopathogenesis of this disease.

(4) This vaccine and adjuvant system are apparently not currently used in humans. Differences in the placentation and immunological transfer between ruminants and humans suggest that a similar presentation is unlikely, but the link between maternal vaccination and fetal/neonatal effect may be an area of potential significance which should be explored.

(5) It is not known whether, following vaccination, some or all cows are positive for a soluble factor (which is presumed to be antibody, a hypothesis supported by preliminary data) in their colostrum. However, based on limited experimental evidence, it seems more likely that a cow factor is more important than a calf factor. While very few cows produce affected calves, when fed "positive" colostrum the majority of calves appear to be susceptible (published data from another research group; Willoughby and Rocchi pers comm.). The underlying pathophysiology requires further elucidation but initial experiments should investigate whether this is a specific antibody or an effect of IgG concentration.

(6) We have considered the risk to humans when ingesting milk from cows which have borne an affected calf. There are 2 areas to consider; whether the soluble factor, presumed to be antibody, will be
absorbed through the human gut, and if such an absorption does occur, whether it will exert an effect, which may be different, in humans. As we have not defined the target of alloimmunity, it is difficult to know whether the hypothesised bovine alloantigen might have a homolog in human cells. And if it does, will the bovine antibody (a) recognize it or (b) be present in sufficient concentration to exert an effect? Further research on the disease in calves may help to define this.

Knowledge transfer

Conferences

1. Association Veterinary Teachers and Research Workers York March 2010 and Nottingham April 2011 A Holliman, S Scholes, S Lambton, Akbar Dasterjdi, C Bell
2. VLA Conference Warwick September 2010 A Holliman, S Scholes
3. British Cattle Veterinary Association Torquay October 2010 A Holliman, S Scholes, A Colloff
4. American Association Veterinary Laboratory Diagnosticians Minnesota November 2010 Sandra Scholes
5. World Buiatrics Congress Santiago Chile November 2010 A Holliman, S Scholes

Practitioner Meetings

1. Dumfries BVA 2010 A Holliman
2. VLA Luddington 2009 G Van der Burgt
3. VLA Penrith 2010 A Holliman
4. VLA Bury 2010 I Mawhinney
5. VLA Thirsk 2010 B Strugnell
6. VLA Langford 2010 A Barlow
7. Shropshire vet society 2010 M Richey.
8. VLA/Western Counties Veterinary Association Clinical Path meeting 2009 F Twomey.
9. Joint Royal Counties and Southern Counties BVA 2010 D Harwood,
10. Borders clinical club 2010 F Howie
11. Cornwall Veterinary Association 2010 A Colloff
12. RVC Emerging diseases meeting R Pearson 2010

Other Meetings

1. Hilary Benn visiting VLA Luddington October 2009 G. Van der Burgt
2. BNP Workshop at European Buiatrics Congress, Marseille December 2009
4. BNP Project team, MRI Edinburgh February, May and October 2010
5. EBLEX/ADAS meeting 2010 M. Richey
6. Food Animal Pathology Discussion Group 2010 F. Howie
7. SE Regional NFU meeting 2010 D Harwood
8. West Midlands Dairy board meeting 2010 A Otter
10. NFU November 2010 D Harwood
13. Hartpury Agricultural College students February 2011 G Van der Burgt
14. Meeting with Pfizer, VMD and Defra January 2011
15. BVA January 2011 D Harwood

References to published material

9. This section should be used to record links (hypertext links where possible) or references to other published material generated by, or relating to this project.
Surveillance: SAC C VS DISEASE SURVEILLANCE REPORT: More cases of ‘bleeding calf syndrome’ diagnosed across Scotland Veterinary Record 2010;167:157-160 doi:10.1136/vr.c3921

Letter: CATTLE HEALTH: Case-control study on bleeding calf syndrome
Andrew Holliman, George Caldow
Veterinary Record 2010;166:406 doi:10.1136/vr.c1664


…… Feature: AVTRW: Coping with the knowledge explosion Veterinary Record 2010;166:638-641 doi:10.1136/vr.c2118


SURVEILLANCE: VLA DISEASE SURVEILLANCE REPORT: Salmonella Typhimurium causes outbreaks of disease in pigs Veterinary Record 2009;165:585-588 doi:10.1136/vr.165.20.585

…… Surveillance: VLA DISEASE SURVEILLANCE REPORT: Congenital abnormalities in calves possibly linked to maternal nutrition Veterinary Record 2010;166:774-777 doi:10.1136/vr.c3080

…… Surveillance: Sac vs Disease Surveillance Report: Outbreaks of idiopathic haemorrhagic diathesis syndrome in young calves Veterinary Record 2009;165:39-42 doi:10.1136/vetrec.165.2.39

…… SURVEILLANCE: SAC CVS DISEASE SURVEILLANCE REPORT: Causes of abortion and stillbirth in cattle in Scotland Veterinary Record 2009;165:677-680 doi:10.1136/vr.165.23.677

…… Letter: Cattle Health: Possible preventive strategy for bovine neonatal pancytopenia
Charlotte R. Bell, Philip R. Scott Morag G. Kerr,Kim Willoughby
Veterinary Record 2010;167:758 doi:10.1136/vr.c6209

News & Reports: VLA/GVS/AGV Conference: Diverging destinies? Comparing the profession in the USA and UK Veterinary Record 2010;167:805-808 doi:10.1136/vr.c6517

Letter: CATTLE HEALTH: Lack of evidence for circovirus involvement in bovine neonatal pancytopenia
Kim Willoughby, Janice Gilray, Maddy Maley,Akbar Dastjerdi, Falko Steinbach, Malcolm Banks, Sandra Scholes, Fiona Howie, Andrew Holliman, Pauline Baird John McKillen
Veterinary Record 2010;166:436-437 doi:10.1136/vr.c1685

…… Surveillance: VLA DISEASE SURVEILLANCE REPORT: Parasitic and tickborne diseases in cattle and sheep in England Veterinary Record 2010;166:66-69 doi:10.1136/vr.c244

Survveillance: VLA DISEASE SURVEILLANCE REPORT: Fasciolosis commonly diagnosed in cattle and sheep Veterinary Record 2010;166:514-517 doi:10.1136/vr.c2060


…… Surveillance: SAC C VS DISEASE SURVEILLANCE REPORT: First cases of pandemic H1N1/09 influenza in Scottish pigs Veterinary Record 2010;166:548-551 doi:10.1136/vr.c2156

Letters: Ruminant Health: Bovine neonatal pancytopenia, and anaemia in lambs caused by feeding cow colostrum
Agnes Winter  
*Veterinary Record* 2011;168:84 doi:10.1136/vr.d370

Surveillance: SAC C VS DISEASE SURVEILLANCE REPORT: Harsh winter prompts concerns about ewe nutrition  *Veterinary Record* 2010;166:674-677 doi:10.1136/vr.c2721

Surveillance: SAC C VS DISEASE SURVEILLANCE REPORT: Significant losses due to fasciolosis in Scottish sheep flocks  *Veterinary Record* 2010;166:382-385 doi:10.1136/vr.c1451

http://oas.services.bmj.com/5c/veterinaryrecord.bmj.com/search/L24/544400957/Top/BMJ/vet-vetoquinol-240111/3418_Alfaxan_Banner.jpg/50686c74773075725465454143645168?x  Surveillance: SAC C VS DISEASE SURVEILLANCE REPORT: Clostridial diseases diagnosed in cattle across Scotland  *Veterinary Record* 2010;166:129-132 doi:10.1136/vr.c300

News and Reports: RESEARCH: Towards a vaccine against *H contortus*  *Veterinary Record* 2010;166:5 doi:10.1136/vr.b5621

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F. Dyer, G. Diesel, S. Cooles, A. Tait  
*Veterinary Record* 2010;167:118-121 doi:10.1136/vr.c3650

]  News & Reports: Research: Research highlights of Moredun's anniversary year  *Veterinary Record* 2011;168:6 doi:10.1136/vr.c7410

...  Surveillance: SAC C VS DISEASE SURVEILLANCE REPORT: Congenital cerebral and cerebellar lesions of unknown aetiology in calves  *Veterinary Record* 2010;167:234-237 doi:10.1136/vr.c4263

**NEWSPAPERS, FARMING PRESS and VLA PUBLICATIONS**

1. Cumberland News October 2010

2. Veterinary Times June 14, 2010 and August 23, 2010

3. Vetro VLA In-house magazine October 2010--role of VLA in the investigation of BNP

4. DairyCo  

**Welcome to DairyCo**

**Bovine neonatal pancytopenia (Bleeding calf syndrome)**

Bovine neonatal pancytopenia (Bleeding calf syndrome) or Idiopathic haemorrhagic diathesis of calves (blood sweating disease)  
5. **Bleeding Calf Syndrome: The Facts**

Also known as idiopathic haemorrhagic diathesis of calves, or blood sweating disease, the cause of this newly identified disease is unknown. The Veterinary Laboratories Agency (VLA) is working with other organisations to investigate this disease.


6.

![Farmers Weekly Interactive](http://www.fwi.co.uk/Articles/2010/06/17/121846/Farmers-Weekly-wins-major-award.htm)

**Making the Farming Connection**

As part of Farmers Weekly's round-up of emerging exotic diseases, Sarah Trickett speaks to Graham David of the *Veterinary Laboratories Agency* about the bleeding calf syndrome.


7. **TheCattleSite Latest News**

http://www.nfuonline.com/

![NFU](http://www.nfuonline.com)

**The Voice of British Farming**

**June 2010 update on Bleeding Calf Syndrome**

01 Jun 2010

**Bovine Neonatal Pancytopaenia (also known as Bleeding Calf Syndrome) – an update**