

## ***Salmonella enterica* serovar Stanley intrauterine infection in a stillborn calf - case report**

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### **Abstract**

The aim of this study was to report the response to a bacterial intrauterine infection in a calf. A stillborn calf, dam's blood and amniotic fluid were submitted for examination. Necropsy of the calf was performed and IgG<sub>1</sub>, IgG<sub>2</sub>, IgM, IL-6 in the calf's serum, IL-6 in the dam's serum, and amniotic fluid were estimated. During necropsy, fluid in pleural and peritoneal cavities stained with haemoglobin and diagonal fissures in the aortic arch endothelium were found. *Salmonella enterica* serovar Stanley was isolated from the spleen, lungs and abomasal fluid. Histopathological examination revealed: inflammatory infiltration and haemorrhages in lungs and small perivascular haemorrhages in the frontal cortex and near the lateral ventricles of the white matter, focal gliosis in the frontal cortex, and neuronal atrophy of the dentate gyrus with diffuse glial cells proliferation in the brain. The concentration of IgG<sub>1</sub> in the calf's serum was increased and IL-6 was detected in both the dam's blood and amniotic fluid. Necropsy, bacterial culture and immunological findings in the stillborn calf confirmed the intrauterine infection with *Salmonella* Stanley as the cause of death. Meanwhile, neonatal diarrhoea (incidence 46%) with high mortality (54%) occurred on the same farm. From diarrhoeic calves, *Salmonella* Typhimurium and *S. Enteritidis* were isolated. Based on available literature this is the first evidence of *Salmonella enterica* serovar Stanley isolation from a stillborn calf.

*Brain injury, Salmonella sp., diarrhoea, immunoglobulin, cattle*

In many countries, the rate of stillborn calves has increased in the last decades. The rate of stillborn calves on Polish dairy farms is estimated between 7.4 and 7.8% (Stefaniak et al. 2011). Stillbirth is commonly defined as the death of a calf at birth or within 24 h after birth, after at least 260 days of gestation (Berglund et al. 2003). The most common causes of stillbirths are perinatal hypoxia and injury, immaturity, intrauterine infection, metabolic disturbances and congenital malformations. Causative diagnosis is difficult and the causes remain unknown in more than 30% of cases (Berglund et al. 2003). The role of intrauterine bacterial infection as a cause of stillbirth in calves is probably underestimated. Intrauterine infection in human foetuses causes brain injury associated with foetal inflammatory response syndrome (FIRS), an inflammatory response that can progress toward multiple organ dysfunction, septic shock and death. Intrauterine infection may lead to the stimulation of cytokine network, which in turn can cause white matter brain damage (Bashiri et al. 2006). White matter damage (WMD) in infants is strongly associated with an adverse outcome. There are two potential, but not mutually exclusive aetiologies of WMD. Firstly, infection/inflammation as part of FIRS (pronounced infiltration of inflammatory cells into the brain and focal microglia/macrophages in response to lipopolysaccharide); secondly, ischaemia-reperfusion (usually no cellular infiltrates are seen and the microglia response is more diffuse).

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In the brains of stillborn calves, subdural as well as periventricular haemorrhages have been considered to be the consequence of foetus ischaemia during delivery (Langanke et al. 1992), but microhaemorrhages may also be present in aborted foetuses in cases of e.g. bovine herpesvirus 1 infection (Brower et al. 2008). The salmonellas are also a well-recognized cause of abortion in cattle, with *Salmonella* Dublin being the most commonly isolated. Nevertheless, other serotypes may be associated with abortion (Hinton 1977; Carpenter et al. 2006). The foetal calf is able to recognize antigens and produce IgM antibody from at least the 90<sup>th</sup> day of gestation and IgG and IgA antibodies from the 111<sup>th</sup> day (Ellis et al. 1978). Sawyer et al. (1973) carried out a quantitative study of IgM and IgG values in the sera of precolostral calves and experimentally and naturally infected foetuses. Immunoglobulins produced by an *in utero* infected bovine foetus significantly exceed the normal level ( $< 0.3$  g Ig/l) determined in healthy, precolostral calves.

The aim of this study was to report the inflammatory response to *Salmonella* Stanley intrauterine infection and brain injury in a stillborn calf.

### Case report

The study was carried out on a stillborn Polish Holstein-Friesian heifer-calf from a twin pregnancy. The percentage of stillborn calves was 7.5% on the 460 head farm not vaccinated against *Salmonella* sp. The cow calved 10 days before the expected term in December 2010 and both calves were dead at delivery. One stillborn calf, dam serum and the amniotic fluid were submitted to the Department of Pathology of Wroclaw University of Environmental and Life Sciences. The amniotic fluid was aspirated from foetal membranes hanging down from the dam's vulva.

During the necropsy of the stillborn calf, transudate fluid stained with haemoglobin was found in the pleural (50–100 ml) and peritoneal (200–250 ml) cavities. The lungs were congenitally collapsed. Diagonal fissures in the aortic arch endothelium were seen with ruptures grossly visible and length from 3 mm to 10 mm. Moreover, haemorrhages had occurred in the adventitia of umbilical blood vessels. The spleen was moderately oedematous. The mucous membrane of the abomasum, the small intestine, both kidneys and suprarenal glands were congested.

During necropsy, samples of lungs and the whole brain (between 6 and 8 h after the calf was born) were taken. Tissue samples were placed in 10% neutral buffered formalin solution and later paraffin embedded, and the sections were stained with haematoxylin-eosin. The brain was sliced in five places according to neuropathological examination schedule. Brain histopathological examination showed that the leptomenigeal blood vessels were hyperaemic and inflammatory cells' infiltration in the adventitia layer was observed. Parietal thrombi were also present in a few leptomenigeal vessels (Plate II, Fig. 1a). Small perivascular haemorrhages were found in the frontal cortex and near the lateral ventricles of the white matter (Plate II, Fig. 1b), as well as focal gliosis in the frontal cortex (Plate II, Fig. 1c) and neuronal atrophy of the dentate gyrus (Plate II, Fig. 1d) with diffuse glial cells proliferation (Plate II, Fig. 1e). Histopathological examination of lungs showed airless pulmonary tissue, inflammatory infiltration and haemorrhages. Both focal and diffuse inflammatory infiltrations were observed (Plate III, Fig. 2).

Sections of inner organs (spleen, lung, liver, kidney) and abomasal fluid were cultured bacteriologically on blood, chocolate, and MacConkey agar plates and brain heart infusion. The culture yielded non-lactose fermenting colonies that were confirmed as *Salmonella* Stanley by biochemical methods and serological typing (PN-EN ISO 6579:2003). A few colonies of *Salmonella* Stanley were detected in the spleen and numerous colonies in the lungs and abomasal fluid; cultures of liver and kidney were negative.

IgG<sub>1</sub>, IgG<sub>2</sub> and IgM immunoglobulins were estimated in the calf's serum by radial

immunodiffusion (Mancini et al. 1965). The concentration of IL-6 in the calf's and the dam's serum and in the amniotic fluid were estimated using ELISA (Thermo Scientific, USA; ELISA was validated to bovine interleukin 6 by the producer). The concentration of IgG<sub>1</sub> in the calf's serum was 0.62 g/l; only a trace of IgM was found, and IgG<sub>2</sub> was not detected. The concentration of IL-6 was 0.215 ng/ml in the calf's serum, 1.62 ng/ml in the dam's serum, and 1.84 ng/ml in the amniotic fluid.

A few days after necropsy we visited this farm due to an outbreak of severe neonatal diarrhoea (incidence 46%) among the calves, with high mortality (54%) despite treatment. During the inspection, presence of large number of birds (mostly pigeons) and traces of rodent faeces were observed. Following samples were taken for bacteriological culture: rectal swabs from four diarrhoeic calves and dam of stillborn calves, from the cloaca of two pigeons, swabs from the feed bunk in lactating and calving cows' pens, swabs from the floor in the calf barn and calving pen, samples of milk replacer and a swab from the mixer wagon. Results from bacteriological examination of samples taken at the farm are shown in Table 1.

Table 1. Results of bacterial culture from samples taken at the dairy farm.

Sample	Isolated bacteria
Rectal swabs from diarrhoeic calves (n = 4)	<i>E. coli</i> (4 cases), <i>Enterococcus</i> spp. (4 cases), <i>Salmonella</i> Typhimurium (2 cases), <i>Salmonella</i> Enteritidis (1 case)
Rectal swab from dam of stillborn calf (n = 1)	<i>E. coli</i> EPEC serotype O111, <i>Staphylococcus aureus</i> and $\beta$ -haemolytic <i>Streptococcus</i>
Cloacal swab from two pigeons (n = 2)	<i>E. coli</i> , <i>Enterococcus</i> spp., <i>Proteus mirabilis</i>
Feed bunk swab (n = 1)	individual colonies of <i>E. coli</i> and <i>Enterococcus</i> spp.
Swab from floor in calving pen (n = 1)	<i>E. coli</i> , <i>Enterococcus</i> spp., <i>Proteus mirabilis</i>
Swab from floor in calf barn (n = 1)	<i>E. coli</i> , <i>Enterococcus</i> spp., <i>Proteus mirabilis</i>
Milk replacer (30 g powder)	no growth
Swab from mixer wagon (n = 1)	<i>Enterococcus</i> spp., <i>Bacillus</i> spp. (individual colonies), <i>Staphylococcus coagulase</i> neg. (individual colonies), <i>E. coli</i> (individual colonies), <i>Proteus mirabilis</i>

## Discussion

The presence of large volumes of transsudate in the pleural and peritoneal cavities might be associated with dystocia. Since the lungs were congenitally collapsed, the calf died before calving. There was no sign of decomposition in the inner organs. An interesting finding was the diagonal fissures in the endothelium of the aortic arch, which are frequently found in calves that died due to Gram-negative infections (Balbierz et al. 1990). Isolation of *S. Stanley* in bacterial cultures confirmed intrauterine infection in this case. Isolation of the bacteria from stillborn calves has not been reported, yet. A case of *Salmonella enterica* serovar Stanley skin infection has been described in a veterinary surgeon who assisted at a difficult delivery (Lazarus et al. 2007). Visser (1991) described two cases of skin infections in veterinarians following delivery of emphysematous dead calves. In the first case, *S. enterica* serovar Virchow was isolated from the human (skin pustules) and from the cow's milk. In the second case, *S. enterica* serovar Dublin was isolated in pure culture from the pustules on the veterinarian's skin and from the cow's vagina and rectum. In both studies described above, bacteriological culture of samples obtained from the calves was not

performed. The presence of FIRS in the calf was indicated by the results of histopathological examination of the brain: inflammatory cells infiltration of leptomenigeal vessels, focal gliosis, and colliquative necrotic foci with microglia cells. However, a large volume of transudate in the pleural and peritoneal cavities, and brain foci of colliquative necrosis may also be associated with hypoxia and brain ischaemia followed by regenerative processes. Lung histopathological findings indicated the presence of severe pneumonia, confirming the presence of intrauterine infection. Therefore, we cannot exclude that hypoxia and brain ischaemia occurred at the same time as intrauterine infection.

The elevated concentration of immunoglobulins in the serum indicated a humoral immune response to antigenic stimulation *in utero*. The IgG exceeded twice the upper level of immunoglobulins in healthy precolostral calves (Sawyer et al. 1973). It is difficult to interpret the IL-6 concentrations in the calf's serum and amniotic fluid, due to the lack of references in cattle, but the concentration in the dam's serum seems normal for this period (Ishikawa et al. 2004).

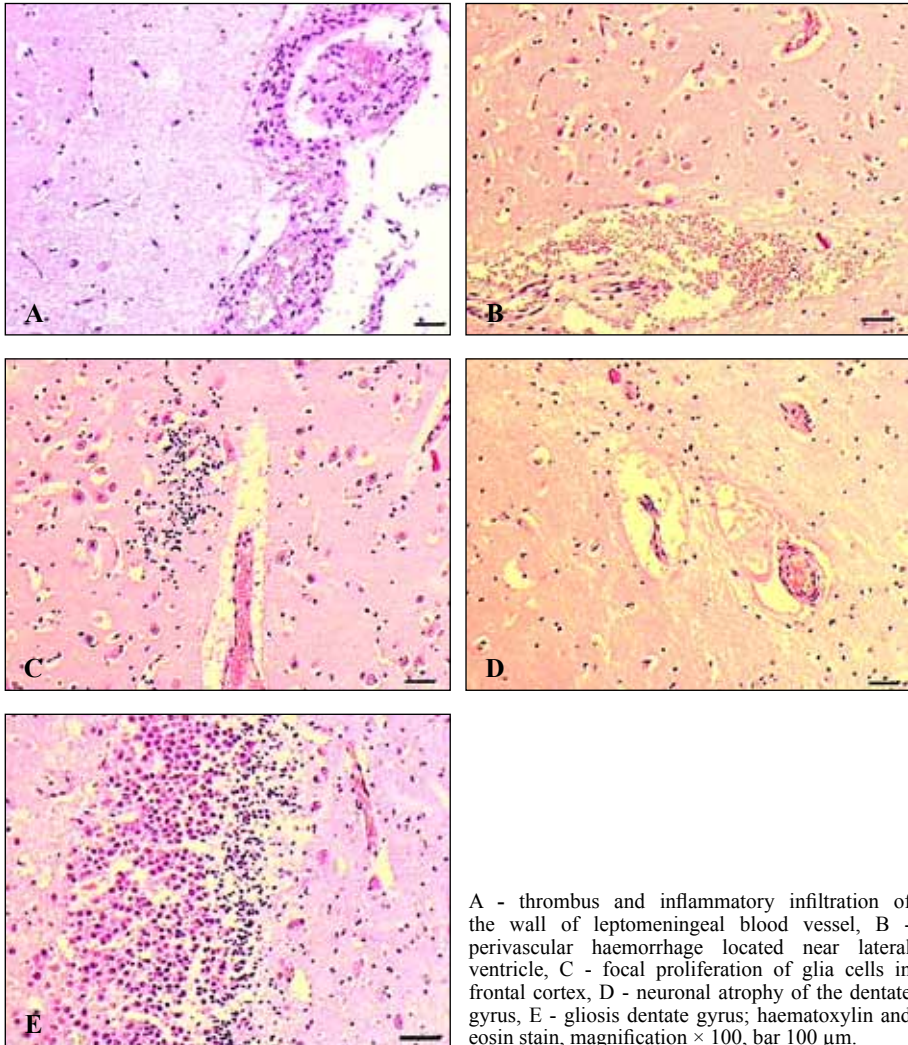
Among bacteria isolated from samples taken on the farm, *Salmonella enterica* subsp. *enterica* serovar Typhimurium and *S. enterica* subsp. *enterica* serovar Enteritidis belonged to undesirable pathological flora. Cattle are considered to be a reservoir of *E. coli* serotype O111 and a source of infection to humans, but the isolated strain was not characterized for the presence of virulence factors; therefore, it is difficult to determine its importance as a threat. *Staphylococcus aureus* may be isolated from rectal swabs from clinically healthy cattle (Dimitracopoulos et al. 1977). Both mentioned microorganisms were isolated from the dam of the stillborn calf. Normal bacterial microflora was isolated from pigeons, feed bunk, the floor in the calving cows' pen, the floor in the calf barn and a the mixer wagon. Isolation of two (or more) different serovars of *Salmonella* on the same farm was described before. In a study by Kirchner et al. (2012) on a dairy farm with *Salmonella* Dublin infection, not only *S. Typhimurium* but other serovars, e.g. Enteritidis, Agama, Newport and Saintpaul were detected. Even though there was no isolation of *Salmonella* from pigeons and *Salmonella* was not detected on the farm before, we cannot exclude the wild birds as a potential source of infection. Other than bird droppings, sources of infection like manure stores or other sick animals could be possible as well (Fossler et al. 2005).

In conclusion, the probable cause of the calf's death was intrauterine infection followed by FIRS and development of brain and meningeal injury. Despite greater perinatal mortality and more common dystocia in twin calves (Sawa et al. 2012), veterinarians should always be aware of the possibility of intrauterine infection. Frequently occurring difficulties in the identification of a causative agent suggest that indirect evidence (presence of FIRS) may be an interesting method of confirming/excluding intrauterine infection as the cause of stillborn or weak calf syndrome. This should increase the detection rate of intrauterine infection cases where the infectious agent was not diagnosed.

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A - thrombus and inflammatory infiltration of the wall of leptomeningeal blood vessel, B - perivascular haemorrhage located near lateral ventricle, C - focal proliferation of glia cells in frontal cortex, D - neuronal atrophy of the dentate gyrus, E - gliosis dentate gyrus; haematoxylin and eosin stain, magnification  $\times 100$ , bar 100  $\mu\text{m}$ .

Fig. 1. Histopathological findings in the brain of a stillborn calf.

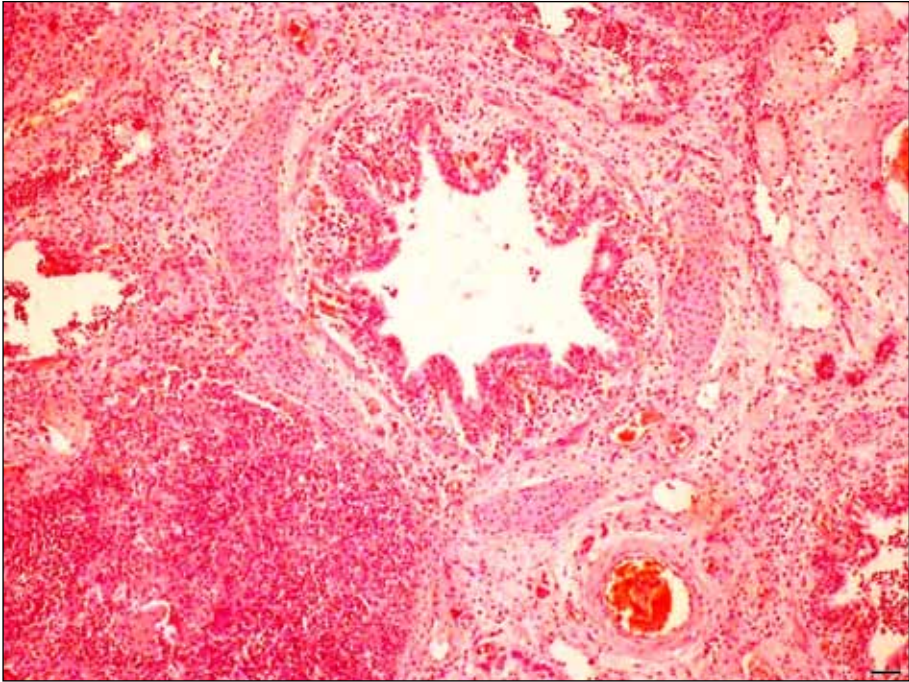


Fig 2. Focal (bottom left corner) and diffuse inflammatory infiltration of airless pulmonary tissue, oedema of the bronchial mucosa of a stillborn calf (haematoxylin and eosin stain, bar 200  $\mu$ m).