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Commonest bacteria identified in calves with arthritis in Norway

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Preface

Norwegian livestock are reputed to have high animal welfare standards. It is the duty of veterinarians to play our part in maintaining these standards but also to identify areas for improvement and continue to strive for the highest standards of animal health and welfare. I chose to study the subject of septic arthritis in calves because I wanted to contribute to evidence-based veterinary medicine and new knowledge on an infectious disease that can have major impacts on the early-life welfare of calves as well as potentially chronic impacts on the welfare of individual animals.

Currently, new research is underway by the Norwegian Veterinary Institute with collaboration with Animalia and NMBU to provide new knowledge on the infectious disease epidemiology of infectious arthritis of lambs managed on Norwegian sheep flocks. By comparison, research into the epidemiology and prevention of infectious arthritis of calves in Norway appears to have been relatively neglected, until now. Given the very limited amount of research into this important issues for calf health and welfare both globally and nationally, I was motivated to be part of a new project that had the potential to contribute new, clinical information on a subject with clear relevance for practicing veterinarians, veterinary pathologists, animal welfare researchers, cattle producers and the wider cattle production industry in Norway.

Summary

Title: Commonest bacteria identified in calves with arthritis in Norway

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Definitions and abbreviations

Arthritis		Inflammation of a joint
Polyarthritis		Inflammation of several joints
Monoarthritis		Inflammation of a single joint
AMR		Antimicrobial resistance
NSAIDs		Non-steroidal anti-inflammatories
AST		Antibiotic susceptibility testing

Introduction

Arthritis is an orthopedic disease that is defined as a disorder of the joint(s). It can present as monoarthritis - only affecting one joint, or as a polyarthritis - affecting multiple joints[1,2.]Arthritis is the general common name given to all types of joint diseases. There are many different types of arthritis and different causes of joint pathology. For example, arthritis can be present as a degenerative joint disease (commonly known as 'osteoarthritis') or as an autoimmune joint disease (known as 'rheumatoid arthritis'). Infectious, also known as 'septic', arthritis, which is the focus of this dissertation, is synonymous with inflammatory changes associated with a bacterial infection of the joint[2].

Arthritis can also be divided into intraarticular inflammations and periarticular inflammations. An intraarticular inflammation is attributed to trauma within the joint capsule[2]. Whereas periarticular inflammation only affects the tissues and structures outside the joint, such as the synovial sheets and the skin without any penetration of the joint cavity[2]. Intraarticular and periarticular inflammation will not be further discussed in this dissertation.

Intraarticular septic arthritis, which from now on only will be referred to as 'arthritis, is the focus of this dissertation. Depending of the severity, duration and localization of the inflammation, this type of arthritis is often associated with a hot, swollen and painful joint, and

most animals will be lame to varying degrees, or in the most severe cases, unable to stand[1,2]. Further discussion of the clinical diagnosis, presentation and treatment will be presented in the following literature review.

The main goal of the treatment of arthritis is to treat active joint infection, minimize synovial inflammation and ultimately regain normal joint function[1],[2]. The mainstay of veterinary treatment is based on antimicrobials (including systemic medicaments) for a relatively long duration and anti-inflammatory drugs[1-3]. There is increasing focus on ensuring minimal but necessary antimicrobial usage (AMU), specifically there is increasing awareness on the type, amount and duration of antibiotics selecting for treating clinical disease in livestock. Good antimicrobial stewardship policies are vital to efforts aimed at minimizing the selection of antimicrobial resistance (AMR). The selection of the most appropriate antibiotic used in the treatment of calf septic arthritis cases can be informed by knowledge on the likely causative agent(s).

In Norway the national veterinary medicines guidelines provide recommendations on the selection of antimicrobial drugs in livestock by veterinarians[4]. These guidelines are stated to "reduce unnecessary and excessive use of antibiotics, to decrease the risk of antimicrobial drug resistance and the preserve the effects of the antimicrobial substances"[4]. The guidelines for septic arthritis state that the infections are most often caused by penicillin-sensitive bacteria,

and advocate that the first choice of antibiotic is benzylpenicillin. Whereas, if an infection associated by penicillin-resistant bacteria is known or suspected, the suggested treatment is systemically trimethoprim-sulphate or gentamicin[4].

A challenge for those developing the national veterinary medicines guidelines is the need for high quality data to inform evidence-based veterinary medicine protocols. Anecdotally, practicing veterinarians in Norway, rarely submit bacterial cultures of the joints of calves with arthritis cases, which provides a limited amount of antimicrobial susceptibility testing (AST) to inform best practice treatment protocols.

The project described here was initiated following discussion between NMBU and TINE with the objective of contributing new knowledge on bacteria commonly identified in the joints of calves with septic arthritis to improve animal health and welfare. This knowledge is relevant to informing best practice veterinary treatment of calf arthritis in Norway. A first step in the process was to conduct a review of the scientific and clinical literature on the topic to provide a background for the studies designed and conducted in Materials and Methods.

Literature review

A review of the scientific literature was performed to firstly, identify the current state of published knowledge on the diagnosis, treatment and prognosis of infectious arthritis cases in calves, and secondly, to identify a knowledge gap on septic arthritis in calves in Norway. The literature search process initially identified a large number of clinical and research articles regarding arthritis in production animals. However, most of the articles that were retrieved focused either on arthritis in older cattle, or in younger animals of a different species, e.g. lambs. Articles found for the literature review was found online, mostly using Oria, searching for terms including “arthritis” or “polyarthritis” + “calf” + “septic / infectious”. The search also included material identified from veterinary clinical textbooks. A summary of the literature review findings are presented below.

Causes of arthritis in calves

Arthritis is often associated with bacterial infection[1-3], that most commonly rises from a hematogenous infection. Umbilical infections (omphalitis) are often one of the most diagnosed diseases in younger calves in the first few weeks of life[3], and from the omphalitis a hematogenous spread of bacteria can manifest in other organs, such as the joints. Omphalitis

may be associated with poor hygiene in and environmental conditions during birth and rearing[2]. Other causes of arthritis include trauma, which can create small wounds overlying and/or connecting with the joint capsule[2]. If the calf acquires a systemic infection early in life, the systemic spread of bacteria will often manifest in the joints[1-3]. It appears to be most likely due to the specific anatomical and physiological properties of articular cartilage within the joints, which are strictly avascular to maintain the mechanical function (no blood vessels run through the cartilage). The vascular supply to the cartilage is provided via small capillaries in the epiphyseal subchondral bone[5]. The small capillaries in the epiphyseal bone resembles hair pin-like structures, as they bend close to the end, near the joint cavity. Here the vessels create sinusoids with lower flow and pressure of the blood, where bacteria easily can be deposited. The anatomy of the synovial membrane lacks a basal membrane, and this facilitates the migration and multiplication of the bacteria.[3,5] Arthritis is not considered to be a congenital condition. Therefore, if neonatal calves present with fixation of the joints appears immediately after birth, deformities such as arthrogryposis would be a likely differential.[2]

Bacterial pathogens associated with septic arthritis

The most common infectious causes of arthritis in cattle, sheep, pigs and horses are considered to be bacterial in origin[2,3,5]. Clinical guidance suggests that the commonest bacteria agents found in calf arthritis cases in Norway are *Staphylococcus* spp., *Streptococcus* spp. and *Escherichia Coli.*[6]. In addition, joint infections associated with *Trueperella pyogenes,*

Salmonella spp., *Mycoplasma bovis* and *Haemophilus somni* are also cited as common bacterial associated with arthritis in young calves[6]. A Canadian study suggested that *T. pyogenes* is the most common bacterial species isolated from young calves (<6 months) with arthritis, followed by *Streptococcus* spp. and Enterobacteriacae[7]. The authors of the Canadian study strongly suspected that *M.bovis* was underdiagnosed because of diagnostic challenges in culture, and suggested that it may be amongst one of the most common causes in Canada.[7]

For calves younger than 4 weeks-old, a common clinical presentation of the polyarthritis can be hematogenous spread from an umbilicus infection[2,3.] Bacteremia can also arise secondarily from other common infections and co-morbidities in young calves, including respiratory tract or intestinal infections[2,3.] Clinical reports suggest that in older calves, it is more common that a single joint is affected, which often starts from periarticular infections (caused by trauma) and thereafter spreads intra-articularly.[2]

Diagnosis of septic arthritis

Clinical examination

The most common clinical findings in cases of septic arthritis are of a severely lame animal with one or more hot, swollen and painful joints[1-3]. During examination of the joint, it is important to check for the presence of any wounds or perforations of the skin overlying key articular structures. The affected calf may be in appropriate or reduced body condition. Cases

often present with elevated rectal temperature (normal range for a calf is 38.5 – 40.0 degrees Celsius), might indicate pyrexia, as well as pain.

Wherever possible, it is always valuable to fully examine the gait of suspected arthritis cases. Further orthopedic examinations should include palpation, flexion and tensioning of the leg[6]. If an apparent wound is visible, sterile preparation of the area, and probing the cavity with sterile gloved hand, and a sterile, blunt object can be done with great caution[6]. The probing can be useful to get information if the wound is either periarticular or intraarticular. If the wound is looking very contaminated, with lots of purulent and necrotic material, probing is not recommended as it can do more damage if it happens to penetrate into the synovial cavity, bringing in external microbial with it and contaminating the wound even more.

Diagnostic imaging

In the field, cost and practicalities limit the use of radiography but it is possible to use radiographs to inform the diagnosis of arthritis and particularly to confirm whether there is penetration into the synovial cavity or not[2,8]. Another more accessible tool is ultrasonography to identify synovial thickening and inflammation[8], although at present this method is anecdotally relatively rarely used in farm clinical practice in Norway.

Arthrocentesis

A sensitive method of diagnosing arthritis that can be performed with minimal equipment is arthrocentesis, which is performed by inserting a needle through the joint capsule into the joint cavity to collect a small sample of joint fluid[1,2]. Bacteriological and cytologic evaluation is considered optimal in order to determine the presence of signs if infection or inflammation[1,2]. A key question for practitioners performing this procedure is whether the animal should be sedated or not during the sampling. According to some veterinary clinical texts[2], sedation is advocated to keep the animal calm (preferably asleep) during the procedure and to reduce the likelihood of the join becoming contaminated during the penetration of the joint space.

The suggested sedative of choice is Diazepam intravenous (IV) 0.1 mg/kg bodyweight[2]. Although, in Norway, the most common sedative selected in cattle is Xylazine (Rompun; Bayer Animal Health), which can be administered by IV at a dosage of 0,016-0,024 mg/kg bodyweight or by the intramuscular (IM) route using a dosage of 0.05-0,3 mg/kg bodyweight.[9] The use of sedative will depend of the condition of the affected animal, but in most cases use of sedative will simplify the process of taking a good and sterile joint sample. A 18G or 16G gauge cannula are recommended to be preferable for taking joint samples from calves with arthritis as these are easier to aspirate purulent and/or viscous content [1,2].

After the animal is sedated, the affected area is clipped bare to the skin, the area is cleaned and aseptically prepared e.g. with Chlorhexidine. Before puncturing the joint and collecting joint fluid it is extremely important to be familiar with and aware of the anatomy of the different joints of the cow fore and hindlimbs[10].

The main limb joint anatomy and anatomical landmarks for arthrocentesis in cattle are:[3,10]

- *The stifle (femoropatellar joint):* consists of 3 joints; the femoropatellar joint, the lateral femorotibial joint and the medial femorotibial joint, where the femoropatellar joint communicates in 60% of the cases with the lateral femorotibial joint, the femoropatellar joint and the medial femorotibial joint always communicates, but there is no direct communication between the lateral and medial femorotibial joints. This concludes that to take a sample from the stifle one can either puncture the aspect on the cranial or the caudal of the collateral ligaments to both the lateral and medial femorotibial joints or penetrate the femoropatellar joint in between the median and the middle patellar ligament, distal of patella.
- *The hock (tarsocrural joint):* consists of 4 joints; the tarsocrural joint, the proximal intertarsal joint (PIJ), the distal intertarsal joint (DIJ) and the tarsometatarsal joint. The tarsocrural and the PIJ always communicates, and the DIJ and tarsometatarsal joint communicates in 30% of the cases. The needle should be inserted between the lateral

collateral ligament and the tendon to the attachment point of *musculus tibialis cranialis.*

- *The carpus*: consists of 3 joints; the antebrachiocarpal (radiocarpal) joint, middle carpal (mediocarpal) joint and carpometacarpal joint. The last two joint are the two most distal joints and they always communicates. In 13 % of the cases they also communicate with the radiocarpal joint. This means the needle should be inserted at the dorsolateral aspect between de lateral collateral ligaments and *musculus radialis extensor*, while flexing the knee.

When the anatomical landmarks are identified, the overlying hair is clipped, and skin site is aseptically prepared, e.g. using Chlorhexidine. The needle is inserted with the bevel facing upwards and a 90 degrees angle at the surface and into the joint. When the joint cavity is punctured in a healthy, non-inflamed joint, a yellow, sticky fluid is aspirated when a sterile syringe is attached to the needle and gentle aspiration is applied[1].

Storage, analysis and interpretation of joint fluid samples

When the sample has been taken it needs to be transferred to a transport medium and can now be stored in a cool place (refrigerator at 4-5 degrees Celsius is optimal) until the sample will be evaluated. Possible investigations include examination of the macroscopic appearance, e.g. watery / thick / cloudy and turbulent looking fluid, measurement of the total and differentiated cell count, total protein (TP) and specific weight/gravity (SG)[1,2]. A diagnosis of arthritis is

supported by findings of increased leucocytes (dominantly, 80-90% neutrophils), increased protein concentration and decreased viscosity. The volume of the joint fluid will also be increased in cases of joint inflammation. If the arthritis is caused by trauma, increased levels of erythrocytes are also commonly identified in joint fluid samples.[2] The aseptically-collected joint sample can also be submitted pre-treatment for bacteriological culture and AST to inform whether antibiotic treatment should be continued or changed according to the results.

Table 1 Interpretation of arthrocentesis sample findings to differentiate between a normal, healthy joint and an infected, inflamed joint (taken from Constable et al.)[2 (p.1409)]

Synovial fluid analysis	**Healthy joint**	**Infected joint**
Macroscopic appearance	*Transparent, clear*	*Turbid, yellow*
Total volume	*Normal*	*Often a marked increase*
Clot formation	*No clot*	*May clot after collection (within minutes)*
Erythrocytes (µL)	*<4.000*	*4.000-8.000*
Leukocytes (µL)	*<250*	*50,000-150,000*
Neutrophils %	*7*	*80-90*
Lymphocytes %	*35-40*	*4-8*
Monocytes %	*45-50*	*1-3*
Microbiology	-	*Presence or bacteria or virus*
Total protein g/dL	*1.2-1.8*	*3.2-4.5*
Relative viscosity	-	*Decreased*
pH	-	*Decreased*

Veterinary treatment of arthritis in calves

Treatment of arthritis can be divided into two different approaches, one medical and one surgical method. In the field, medical treatment is by far the most used since it is the most cost-effective, less time-consuming and it requires basic equipment, as compared to the more expensive and demanding surgical approaches.

Medical treatment

Principles for treatment of septic arthritis are to control the infection, largely by using parenteral antibiotic therapy, to drain the synovial cavity of infected fluid as far as possible, control the inflammatory reaction and to recover normal joint function as soon as possible.[1-3,6] For example, the Norwegian school of Veterinary Medicine (NMBU Adamstuen) have long recommended this therapy plan as a part of their hospital-referral treatment:[6]

1) Lavage of the infected joint by sterile flushing with 0.9 % (NaCl) saline or Ringer's lactate solution, with two different points of joint punctuation and ports (one ingress and one egress).
2) Complete flushing by deposing an intra-articular injection of 5.000.000 -10.000.000 IE of crystalline penicillin.
3) Systemic antibiotic treatment for 10-14 days.
4) Reduction of pain associated with joint inflammation through administration of non-steroidal anti-inflammatory drugs (NSAIDs)

5) Where appropriate for the case (especially inflammations of the carpus) it is possible to bandage the joint, to prevent further infection via the puncture sites to stabilize the joint and to secure rest for the leg while recovery is expected.
6) Optimal care and attention during the recovery phase, including rest, is considered to be an important part of the therapy plan through restricted exercise e.g. a dry, warm and clean pen.

Surgical treatment

A more expensive and potentially more effective treatment is the use of arthroscopy to provide clear imaging of the internal joint, by inspecting the joint cartilage, joint space and joint capsule[10]. The costs of this surgical procedure and equipment and sterility required mean this is prohibitive for field use and is very rarely used as a treatment for calves suffering from arthritis albeit in the referral hospital setting.[10,11]

Prognosis

The prognosis is influenced by several factors including timing of the treatment, sterile environments during the lavage, cause of arthritis, antibiotic selected and the severity of synovial inflammation that has occurred.[2] The prognosis is reduced in cases where more than one joint is affected. More aggressive treatment plans may be required to treat polyarthritis. It is also suggested that repetitive joint flushing several times, with an interval of 24 hours, for up

to 3 days, is recommended for more severe cases[6]. If a penetrating skin wound is found to connect with the joint space, the prognosis is very poor, but it can be treated with success if the therapy is started soon enough. Severe cases, with a lack of clinical response, e.g. extensive joint swelling remains, chronic lameness, severely reduced joint mobility, and muscular atrophy, or more than one joint involved carries a poorer prognosis. Euthanasia is a treatment option and on animal welfare grounds, chronic cases and those not showing considerable clinical improvement should be promptly euthanised[1,2].

Herd preventive management strategies

Joint infections might be prevented by optimal calving and rearing hygiene practices[12]. The risk of the umbilicus acting as a portal for bacterial infection may be reduced through general hygienic and antiseptic measures that should be taken before, during and after birth. Some sources also recommend topical disinfecting the umbilicus of neonatal calves[2(Perinatal Diseases, p.1877-1879),12]. Whilst this is a widely recommended practice, no specific evidence was found in the literature about the efficacy of this as a preventive tool against joint infection in calves.

Greater research on septic arthritis has been performed in the equine veterinary field, where it is known that foals that have not ingested sufficient high quality colostrum within the first 4 hours after births have a higher risk of developing arthritis.[5] It is likely that poor colostrum intake is a risk for bacterial infection and arthritis in calves. Optimal care and feeding of the

pregnant cow and ensuring that the newborn calf receives a sufficient quantity of good quality colostrum is also key to the development of a good calf immunity and resistance to local diseases on the farm. In Norway, a good quality of colostrum is defined as immunoglobulins level exceeds 50 g/L of milk, and current industry guidelines suggest that calves should receive more than 8,5% of their bodyweight within the first two hours of birth[13].

Veterinary Hospital Data as a tool for research into calf arthritis

Currently, research into the septic arthritis in calves is limited. The limited, available scientific evidence on causes, diagnosis, treatment and prognosis of septic arthritis in calves was largely based on the analysis of veterinary hospital records from 1) Montreal, Canada, and 2) Shaherkord, Iran, and provides most of the current evidence discussed here.

The Canadian study[7] was a retrospective study of 64 cases of arthritis diagnosed in calves younger than 6 months during 2009 to 2014. The study identified the most common affected joints to be the carpal joint, followed by the stifle and tarsus (hock). Isolates of *Catalase-negative Streptococcus* spp. and *Mycoplasma bovis* were the most common bacteria found.

The veterinarians in the Canadian hospital-based study routinely submitted arthrocentesis samples for *M.bovis* culture but there are also challenges with the detection of *Mycoplasma* spp. during laboratory culture[7].

The Iranian study[14] followed a slightly small population of 40 calves from newborn up to 3 months old. Both blood and joint fluid samples were cultured to assess for bacterial growth. The most common bacterial findings in both blood and fluid samples were *Staphylococcus aureus, Corynebacterium bovis* and *Escherichia coli*. In 8 cases (20%) they diagnosed *M. bovis*, and in 4 of these 8 cases (10%), the calves were also had clinical signs of pneumonia. The most common used antibiotics used to treat the calves in Iran was gentamycin, which is not a first-line antibiotic of choice for cattle in Norway.[4]

Prevalence of septic arthritis in Norwegian calves

Currently, the true prevalence of arthritis in calves aged six months and under that are both diagnosed and/or treated in Norway is unknown. The best estimate of disease prevalence come from two main national databases that collect standardized data on cattle health and disease. Firstly, cattle syndromic disease diagnoses that are voluntarily registered by producers in the cattle health registration system (reported and published by Animalia and TINE Rådgiving)[15.] Secondly, the compulsory electronic veterinary medicines registrations (*Dyrehelseportale*n) that are completed by Norwegian veterinarians every time they prescribe and administer any veterinary medicines, including antibiotics (to production animal species). At present, these databases capture registrations on three codes (Table 2) that both producers and veterinarians can use to record arthritis in an individual animal.

Table 2: National codes for the registration of arthritis in livestock species

Code	Diagnosis	Description
362	Joint diseases in animals younger than 1 month	*Arthritis in newborn - young*
363	Joint disease in animals between 1-6 months old	*Arthritis in young animals*
364	Joint disease in animals older than 6 months	*Arthritis in growing animals*

Septic arthritis in calves aged six months and under was the focus of this study i.e. national disease codes 362 and 363. Therefore, code 364 (Table 2) will not be discussed further here.

The diagnoses registered in the cattle health recording database (see Table 3 for examples) are presented in the annual report as numbers of recordings in males, in heifers and heifer calves, and recordings in dairy calves[15]. From this data it not possible to identify disease prevalence in production animals younger than 6-month-old. Although, it is very likely that, young animals are represented in the group of males and heifer/heifer calf category.

Table 3 Commonest disease recorded in the annual cattle disease registrations for 2017-2018 in male animals, heifers and heifer calves in number and percentage (%) recorded

(Data taken from Helsekortordningen Storfe 2018)[15]

Code	**Disease**	**Number and percentage (%) of cases recordings in male cattle by year**		**Number and percentage (%) of cases recording in heifers and heifer calves**	
		2017	**2018**	**2017**	**2018**
251	Diseases of the respiratory tract - unspecific	3368 (26%)	3562 (27%)	2876 (16%)	3029 (16%)
263-265	Gastritis/Enteritis	2113 (16%)	(1997/15%)	2107 (12%)	2165 (12%)
362-364	Arthritis	1859 (14%)	1937 (15%)	1434 (8%)	1436 (8%)
294	Umbilical infection	438 (3%)	560/ (4%)	247 (1%)	379 (2%)
Total number recorded		12 866	12 944	17 592	18 366

The most recent data from the national cattle health register (Table 3) supports earlier data findings that suggests that in Norwegian cattle the four most common diseases in calves are divided between respiratory diseases (46%), gastritis/enteritis (26%), arthritis (24%) and umbilical infections (4%).[16] There are, however, some limitations with estimating disease prevalence based on these registrations. For example, a female cow will be categorized in the group “heifer and heifer calves” until the day she delivers her first calf, and a male animal will

be categorized in the "males" section until the day that the calf is slaughtered. Furthermore, the same animal can be registered several times for the same disease if the veterinarian makes several visits during one episode of illness for treatment. In this case, the same case can be appearing in the register as several cases, rather than a single case undergoing repeated treatment.

Current veterinary medicine guidelines for calf arthritis in Norway

Arthritis in calves appears to be a relatively common health and welfare issues in Norway today. According to the national veterinary medicine's guidelines in Norway[4] the first-line antibiotic for treatment of cases of septic arthritis is benzylpenicillin, when penicillin-sensitive bacteria (e.g. *Streptococcus* spp and *Staphylococcus* spp.) are suspected. If penicillin-resistant bacteria are suspected (e.g. *Escherichia Coli*) to be involved at the primary infective agent, then systemic treatment with trimethoprim-sulphate or gentamicin is advised[4]. In guidance provided to veterinary students of NMBU, *T. pyogenes* was mentioned as the most common bacterial agent identified in calf arthritis cases[6].

Identifying a Knowledge Gap in the Literature

Globally, research into septic arthritis in calves is limited to analysis of clinical data from relatively small populations of calves examined and treated in two veterinary hospital settings. We lack current knowledge on the commonest bacterial species cultured from calves with one or more infected joints. New knowledge on the commonest bacterial agents found in calves aged six months-old and younger requires aseptic sampling of a clinically-affected joint to obtain a sample of joint fluid for bacteriological testing and, ideally, also antibiotic susceptibility testing. However, this practice, known as arthrocentesis, is currently very rarely performed by veterinarians in Norway when treating a calf with arthritis. This might relate to the lack of familiarity with the technique, and/or the cost of bacteriological testing. The lack of knowledge identified in the literature relates to a knowledge gap of clinical relevance in order to inform effective and evidence-based veterinary decisions regarding antibiotic selection in the treatment of calf arthritis in Norway.

Pre-treatment leads to misguided and not-representative sampling of the affected joint. In the future, arthrocentesis might become a standard clinical procedure when treating animals with suspected bacterial infection, and not just limited to sampling of cases that appear refractory to treatment or the first antibiotic selected.

The author found no scientific studies reporting the diagnosis, treatment or prognosis for arthritis in Norwegian calves. Most of the data and reports identified pertained to clinical reports and anecdotal observations that were not supported by clear evidence or data. In contrast, there has been previous research into the common causes of septic arthritis in calves in based on veterinary hospital registrations made in Canada[7], and Iran[14]. Based on the methods and findings reported in those studies, it was proposed that a retrospective analysis of veterinary hospital data from Norway, further informed by a prospective study using veterinary submissions of joint fluid samples from cases in Norway, would contribute much-needed new research on the subject.

Aim of study

A knowledge gap on the common bacterial agents cultured from calves with septic arthritis in Norway was identified through a scientific literature research.

The main aim of this study was to **describe the most common bacterial agents identified in septic arthritis cases in Norwegian calves aged six months or younger**.

A secondary aim was for this new knowledge and data to be used to assess whether national veterinary treatment guidelines ("*Terapianbefaling*")[4] on the selection of first-line antibiotics were still appropriate for the treatment of calves with arthritis.

Material and Methods

The literature review identified a need to fill an important knowledge gap on the commonest bacteria identified in joints of calves with septic arthritis in Norway. The literature review informed the design of two studies presented here:

1. A descriptive study based on analysis of retrospective clinical and pathological records (2014-2018) on septic arthritis cases and necropsies examined at NMBU Faculty of Veterinary Medicine.
2. A prospective cross-sectional study based on veterinary submissions of joint fluid from affected calves during summer 2019.

The aim of the studies described here was to describe the number and proportion of bacterial spp. identified in the joints of calves aged six-months old and young with septic arthritis in Norway. This new data was key to maintaining good veterinary antimicrobial stewardship and informing 'best practice' diagnostic and treatment guidelines for veterinarians in Norway

Study 1 - Retrospective analysis of septic arthritis cases referral and necropsy cases

Study design

A descriptive study was designed and conducted based on analysis of retrospective data from clinical and necropsy diagnoses of calf septic arthritis. Data were captured from three different clinico-pathological sources including registrations from live and dead submissions of calves aged six months and under presenting to the at NMBU Veterinærhøgskolen, campus Adamstuen, Oslo.

Data were captured in the form of case records and electronic journal entries from the;

1. Farm animal referral hospital (*Stasjonærklinikken*)
2. Farm animal clinical practice (*Ambulatorisk*)
3. Veterinary pathology section (*Patologen*)

Veterinary clinical journal records

All journal cases of the NMBU farm animal hospital and clinical practice from 2001-2018 including both paper records and electronic registrations on the ProfVet recording system were released to the researcher. For this study, five years of data; from 1 January 2014 to 31 December 2018 was analyzed, with recordings including cattle younger than 6 months old, and

at some point diagnosed with arthritis in the hock, stifle and carpus. The methods of data capture used by NMBU did not provide an overview of the number of cases submitted during the study.

Veterinary pathology records

A database was released to the researcher from the veterinary pathology section containing all registered bovine arthritis cases (including adult, growing and young calves) they had reported during the last five years, with an either diagnosed or suspected septic arthritis. The search terms "*polyarthritis"* or "*arthritis" AND* "*cattle"* were applied to the BasAmPro database of veterinary pathological reports for 1 January 2014 to 31 December 2018. The records were manually searched as previously described to capture cases meeting the inclusion criteria.

Database management

Data were checked manually to exclude cattle cases that did not meet the study age criterion. Individual digital clinical records were visually assessed and examined in detail in turn to check the diagnosis of arthritis, and to record arthrocentesis, and results bacteriological and culture testing (Appendix 1). Identifying details such as producer information or clinician identity were not recorded in the database to preserve anonymity.

Inclusion criteria for a septic arthritis case were all of the following:

- Species: cattle (calf)

- Age: six months old/180 days or under
- Recorded clinical signs of arthritis: at least one inflamed joint (including carpus/knee, tarsus/hock or the stifle and/or any combination of these joints in cases of polyarthritis)

Exclusion criteria for this project were:

- The calf was already under antibiotic treatment before the joint sample was taken – excluded since this give false negative or inaccurate bacteriology results.
- If no signs of ante-mortem signs of arthritis were registered in the case notes/history.

All the relevant cases were manually entered and save in an Excel file (Microsoft Office), where the cases were categorized based on the pre-given journal number, calf age, arthritis diagnosis (anatomical location of the joint(s) affected), collection of bacteriological samples, recording of bacterial growth (including contamination or mixed growth) and bacteriological findings (negative or specific bacterial growth).

Data analysis

The aim of analysis was to describe the frequency and percentage of calves affected by arthritis, and to describe bacteriological sampling and culture results. Data was mainly in count (number) and binary scores (yes/no or 0/1) and was descriptively explored using Excel (Microsoft Windows). Data was also visually examined using bar charts and pie-charts.

Study 2 - Arthrocentesis samples submitted by practicing veterinarians

Study design

A major dairy company in Norway (TINE) were approached for support with veterinary contact and identifying herds registering high levels of arthritis registrations in calves (codes 362 and 363). However, due to difficulties with access to the data and general data protection regulations (GDPR) regarding release of veterinary email details for contact, neither TINE nor Animalia Kukontrollen were able to release this data for analysis.

An alternative recruitment strategy was therefore based on purposeful sampling, in which we targeted veterinarians known to NMBU Sandnes and personal contact with the research team, including those who had previously submitted ovine clinical and pathological samples, and/or supported other diagnostic sampling projects. Veterinarians were contacted via email and/or telephone contact. We also used a snowball sampling approach, asking interested veterinarians who got in contact with the researcher (by email or phone) and requested further information and a sampling kit to suggest other potential colleagues that might be willing to participate. In addition, to capture an even greater cross-section of veterinarians, an open advert was placed on the Production Animal Veterinarian Facebook group with a membership of over 1600 vets to contact the author for further details (Appendix 2).

Joint sampling protocol

Sampling kits including two charcoal swabs, sterile gloves, sterile gauze swabs, an addressed return envelope, and short diagnostic submission form (Appendix 1) and a "How-to-take-a-joint-sample" instruction sheet (Appendix 3) and was offered to practicing veterinarians from 1 March to 31 August 2019. Briefly, veterinarians were asked to submit pre-treatment aseptic joint samples, and to complete a short form to capture data on the case (age, breed, clinical signs), clinical signs (categorical tick-the-box), and treatment administered (drug type, dose and duration). The "how to" form was included in the sampling kit in order to encourage a standardized method for sampling in the field, optimize the sterile conditions for sampling, and provide practical information for those less confident or unfamiliar with arthrocentesis.

Bacteriological analysis and antimicrobial susceptibility testing

All bacteriological submissions were to be sent by post and analyzed free-of-charge by NMBU Sandnes bacteriological laboratory free-of-charge (usually charged at 300 NOK per sample). Results were expected to be reported by telephone/email to submitting veterinarians within 48 hours of sample receipt at NMBU Sandnes.

Data analysis

The aim of data analysis was to describe the age of calves presenting for treatment, antibiotic choices of veterinarians and the frequency and any patterns in bacterial species cultured.

Results

Study 1 - Retrospective analysis of clinico-pathological data

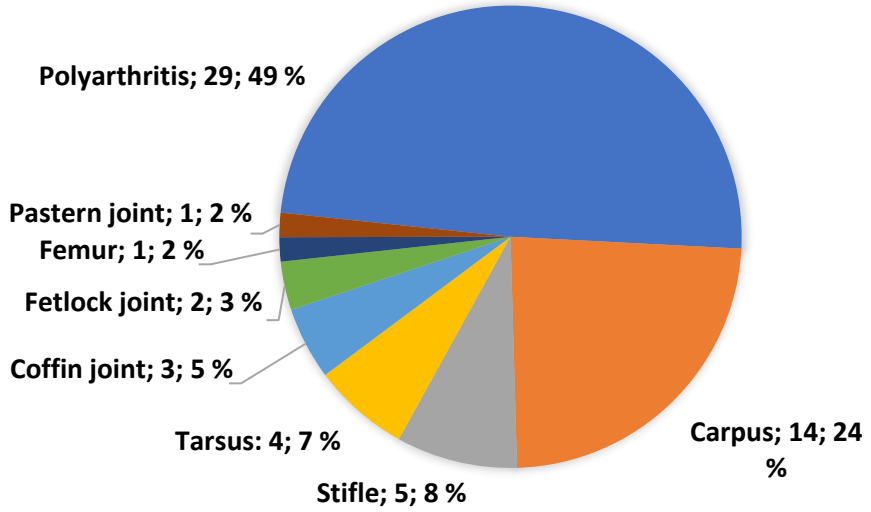
Clinico-pathologial registrations

Of the total of 463 bovine arthritis cases, 59 case records (12.7%) represented calves referred to the NMBU farm animal hospital between 1 January 2014 to 31 December 2018 and matched the study inclusion criteria. All of these 59 cases were submitted for necropsy at the veterinary pathology section. No cases of septic arthritis were identified from examination of the manual or electronic NMBU farm animal clinical practice records. The 59 cases represented 19 calves that were 30 days or younger (32%), and 40 calves that were classified between one to six months-old (68%) Further categorization of the age groups to 0-40 days, and 41-180 days, identified that 30 of calves diagnosed with at least one joint with septic arthritis were between the age of 0-40 days when examined at necropsy, and 29 calves were between 41-180 days.

Most of the cases examined at the hospital were treated (n=52; 88%) for arthritis. A few were not treated but were examined and euthanised on animal welfare grounds (n=7; 12%). Data mining of text entries in the clinical records indicated that treatment varied from washing and disinfecting overlying skin wounds, concurrent to systemic antibiotic treatment of septic arthritis. Out of the 52 calves that were treated, 12 (23%) were treated with systemic antibiotics

(after joint sampling), but 40 calves (77%) receiving treatment were not treated with antibiotics. Intramuscular benzylpenicillin (Penovet vet. Boehringer Ingelheim) appeared to be the first antibiotic of choice and used in 11 of the 12 cases (92%) receiving antibiotic treatment. Trimethoprim-sulfaphate (Borgal vet. Ceva Santé Animale) was selected as the first antibiotic of choice in one calf. In total, 38 joint fluid samples (representing 38 cases) were submitted for bacteriology. No AST results were found in either clinical nor pathological journals. Records indicate that a joint sample was taken from 14 calves before euthanasia, but the most samples (24) were collected at necropsy.

Figure 1 The percentage (%) distribution of affected limb joints in 59 calves diagnosed with septic arthritis at NMBU Faculty of Veterinary Medicine



As illustrated in Figure 1, roughly half of all calf cases (n=30; 51%) were diagnosed with a single joint infection and the other half were diagnosed with polyarthritis (n=29; 49%). In cases where a single joint was affected, the most commonly affected was the carpal joint, followed by the stifle and third most common was the tarsus (hock) joint (Figure 1).

For the 59 calves, bacteriology findings were recorded for 38 cases (64%). In 21 cases (36%) no clinical details on arthrocentesis, bacteriology nor AST results were recorded. The commonest finding (in 8/38) was no bacterial growth (21%) in joint fluid samples (Figure 2). Where bacteria growth was detected, *T. pyogenes* was the most common species cultured (Figure 2). There were also six cases that were interpreted as 'contaminated' where mixed growth of *E.coli, Enterococcus* spp. and *Proteus* spp. (fecal-associated bacteria) were cultured.

Figure 2 Bacteriology results of 38 joint fluid samples from calves with septic arthritis

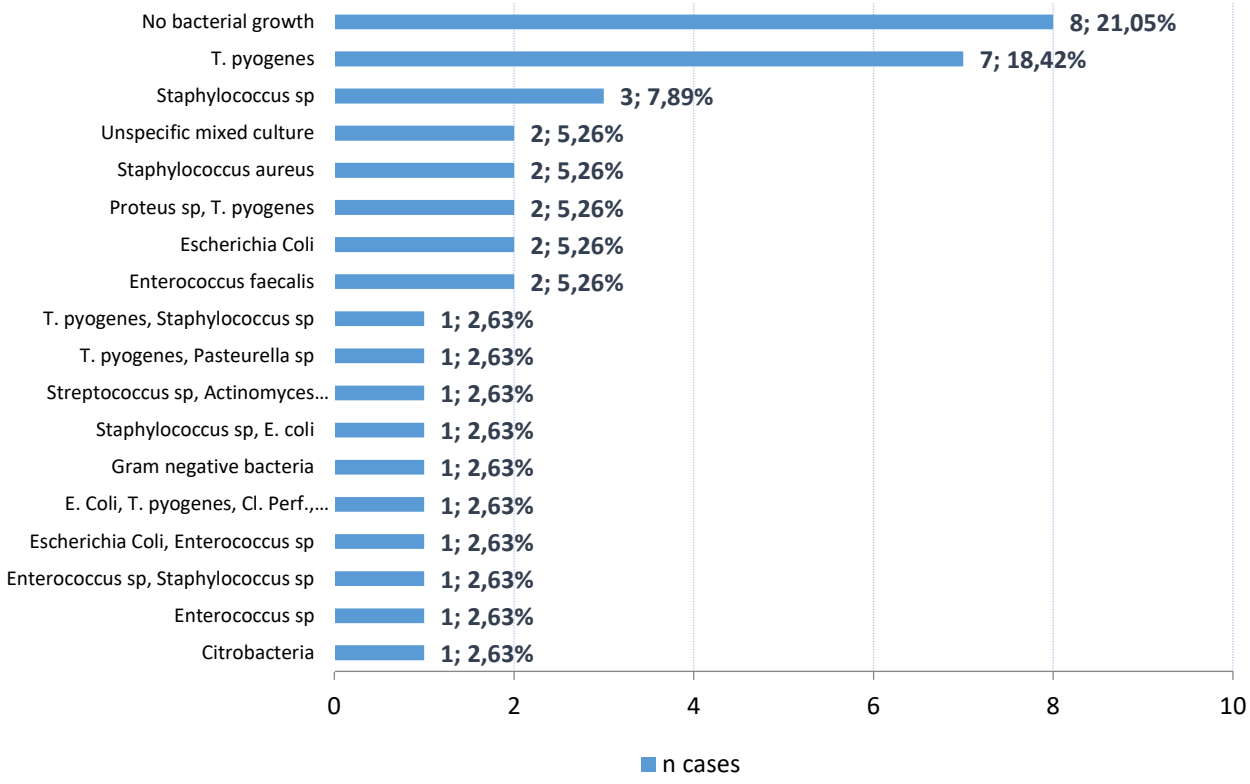


Figure 2 illustrates that *T. pyogenes* was found in single or mixed culture in a total of 12 joint fluid samples (32%), followed by *Staphylococcus* spp. (included *S. aureus*) in 8 (21%) samples. *Enterococcus* spp. (included *E. faecalis*) was the third most commonly identified (n=6;16%), followed by a pure growth of *E. coli* (n=2, 5%). Some bacterial spp. were only found in single samples, such as *Streptococcus* spp., *Pasteurella* spp. and *Clostridia perfringens*.

No bacteria were cultured in 21 % of samples (n=8). A single pure bacteria growth was identified in 50% of cases where joint samples were collected (n=19). Mixed growths of two bacterial species (n=8) in 21%, and three or more different species in 8% (n=3) of samples.

Study 2 – Arthrocentesis samples submitted by practicing veterinarians

Veterinary submissions

Sampling kits were sent to 22 veterinarians across the different regions of Norway, which if all used, gave potential for a total of 44 joint fluid sample submissions. During 1 March to 31 August 2019 only a single pre-treatment sample was received by NMBU Sandnes that had been taken from the carpus of a calf aged less than six months-old with clinical signs of arthritis. Bacteriological testing identified a highly mixed bacterial growth, consisting of mostly coliform and other colonies, which likely indicated a contaminated growth during joint sampling. Therefore, further diagnostic testing, bacterial identification and AST were not performed by the laboratory.

Discussion

To the author's knowledge this is the first study to describe the commonest bacteria identified in calf arthritis cases investigated in a veterinary school in Norway during 2014-2018. A total of 59 cases were captured, which appears to be a relatively small sample, but is similar to the larger Canadian study[7], that followed 64 cases over a longer period of time. Referral hospital cases represent biased populations and the findings are specific to a small geographical region (Oslo area), and includes producers that were willing to submit calves for examination and necropsy. Therefore, the generalizability of this study is limited and it is recognized that the calf population captured here might not be representative for all of Norway. However, this is the first study to examine data on the common bacterial pathogens identified in calves with arthritis in Norway. Further studies consider including a larger study and a field-based population, but would require specific funding to support the time, costs and labor that additional on-farm and veterinary practice and hospital-based sampling would consume.

Database management including manual data checking and cleaning of clinical records, which required a considerable amount of researcher time. A total of 463 cases were reduced down through manual data mining of the records, which required extensive reading and close examination of case records. The process identified that 59 cases reached the study case definition. There is potential for improvement here with the use of electronic clinical and

pathological records used by NMBU Faculty of Veterinary Medicine and to improve the accessibility of such data to inform evidence-based veterinary medicine.

The quality of the records provided was variable. Clinical records were, at times, confusing and often lacked systematic recording of important clinical and diagnostic details. For example, some records lacked background information, such as the age of the calf, and breed. Others clinical journals indicated that joint fluid sampling was performed but the bacterial growth results were not maintained within the file.

Results from AST were not found any of the clinical records and may indicate that testing for antibiotic resistance isn't always routinely performed. Alternatively, this might represent a different system in recording and storage of AST results. There were only four joint samples that were taken while the calf was alive. The remaining 34 samples were taken during post-mortem examination. This suggests that joint sampling for bacteriology and AST isn't routinely performed in the NMBU farm animal hospital practice, or might imply that incoming referral calves are more often euthanized. It is possible that clinicians elect for sampling to be conducted in euthanized animals to reduce the need for invasive diagnostic sampling into a painful joint. Furthermore, results from live animal were unlikely to inform antibiotic selection for that specific given than most calves were euthanized following examination.

Bacterial samples captured from internal organ samples rather than the direct sampling of inflamed joints might result in a diagnosis of multisystemic septicemia including signs of arthritis. However, these data were not included in the bacterial findings here since no direct bacteriological sampling of the joint was conducted. Therefore, there was likely more septic arthritis cases that could have been included, but the lack of specific sampling and/or poor maintenance of journal records precluded this. Mostly electronic records were used, but some of these records (especially from pathology) still had to be printed to provide a paper format for ease of data record checking when searching clinical journals.

These results indicate that the carpus, stifle and the hock are commonest joints diagnosed with septic arthritis. This concurs with findings from other veterinary referral hospitals. Just over half of these calves had one or more than one affected joint in calves younger than 40 days, which suggests that the calves were most commonly diagnosed with arthritis in their first month of life. This can suggest that the calves are infected in an early stage of their life. Speculatively, the infection is most likely occurring in the first couple of weeks to the first month of life. It is also likely that there is a delay between infection and inflammatory changes result in clinical signs that are observed by the farmer. Co-morbidities were not investigated in this study and it was not identified through examination of the clinical records whether potential contributing factors such as concomitant omphalitis and/or deficiency of maternal immunoglobulins[16] were present in the young calves included in this study.

The findings agree with clinical advice on calf arthritis in Norway. The most common bacteria cultured in septic arthritis cases in calves in Norway aged six months-old or younger were (by order) *T. pyogenes, Staphylococcus* spp. and *E. coli.* In spite of the lack of AST results in case files, national guidelines suggest that in most cases the selection of benzylpenicillin as the first choice antibiotic is appropriate for cases associated with *T. pyogenes* and *Staphylococcus* spp. infection[4]. However, in infectious arthritis cases associated with *E. coli* or *Enterococcus* spp. other antibiotics would be indicated (e.g. trimethoprim sulphate or gentamycin[4]). These findings support the diagnostic value of pre-treatment arthrocentesis to inform changes in the first antibiotic of choice selected, based on pre-treatment bacteriology culture and AST results.

The findings do show some similarities to the findings in other studies (e.g the Canadian study[7]) where *T. pyogenes* wad described as the most common bacteria in arthritis as well. However, they also found that *M. bovis* was very often found in arthritis, and due the lack of testing of these bacteria in Norway it's not possible to say in which amount (if any), *M. bovis* is existing in arthritis in Norwegian calves. Also, due the lack of comparable studies found it's hard to compare our Norwegian study to the international ones and make a statement if the prevalence of bacteria found is somehow similar to other countries or not.

Based on the number of testing kits and information provided, there was potential to receive 44 field samples from cases across Norway. The very poor submission response from veterinary practitioners across Norway was disappointing given the recruitment drive and providing the necessary equipment and free-of-charge testing. Furthermore, the single sample that was received was relatively low value to the study results and was considered to be contaminated. This may be due to contamination of the sample with skin bacterial contaminants due to sub-optimal aseptic skin preparation, or sampling technique. Although, it is not possible to exclude the fact this might also reflect a true mixed-growth bacterial infection of the joint.

Follow-up was contact made with seven veterinarians who received a sampling kits but did not submit joint fluid samples. Four of the seven reported that the lack of submissions reflected their lack of clinical diagnoses of any calves with arthritis during the free laboratory testing period. This suggests that future research of this kind could consider offering a longer period of time, with additional reminders to veterinarians to increase the possibility of sample submission, as well as including a larger number of veterinarians than here.

This study provides novel data on the commonest bacterial species identified in the joints of calves with septic arthritis in Norway. The topic is ripe for further funding for a larger project. Study experiences highlights the need for further consideration into the recruitment of clinical veterinarians in Norway to undertake diagnostic sampling in the field. The lack of samples

submitted might reflect the lack of familiarity and inexperience with performing arthrocentesis, or the six month period of sampling that might have been too short. Here qualitative research methods, for example using interviews or focus groups to better understand veterinarian decision-making on diagnosis and treatment of septic arthritis, might support improved targeting strategies and veterinary support in future research programs. Further research could also employ a full-time researcher to capture field samples or provide financial reimbursement to encouraging veterinarians to take pre-treatment samples, in order to capture a wider geographical area and variety of clinical cases.

Conclusion

Calves in Norway aged six-months and under, diagnosed with arthritis and submitted to NMBU Faculty of Veterinary Medicine, were most commonly identified with joint infections associated with *T. pyogenes*, followed by *Staphylococcus* spp., *E.coli* and *Enterococcus* spp. These findings support current veterinary medicines guidelines in Norway indicating that benzylpenicillin is the most appropriate first line antibiotic choice. However, there were a few cases, where pre-existing AST knowledge suggested that penicillin would not be the first antibiotic of choice, and highlights the benefits of pre-treatment arthrocentesis for informing secondary treatment decisions. Whilst it seems likely that bacteria cultured in the joints of calves pre-treatment were associated with infection and joint pathology, this study has not

identified that the bacteria identified were the cause of arthritis. The data presented here represents a relatively small and biased population of hospital referral cases, including those unresponsive to treatment and submitted for necropsy. Despite this, it demonstrates the value of analysing routine farm animal referral hospital and pathology records as a means of describing frequencies or patterns in specific disease syndromes and in patient data. There is greater opportunity for further analysis of routinely-captured retrospective veterinary hospital data to inform improved clinical practice and support evidence-based veterinary medicine. Study experiences indicate there is room for improvement in the quality and type of data recorded by clinicians and pathologists at NMBU Faculty of Veterinary Medicine. For example, a standardized electronic input system for both clinical and pathology cases that captures background case information (age, breed, reason for submission) could reduce missing data and electronic transfer of laboratory results could reduce the loss of valuable records, such as bacteriology and AST results. Improved electronic search options to improve the accessibility and analyzability of veterinary hospital case records, and permit use of statistical data and text mining programs could also reduce the time and labour required for manual searches through paper case files. It is unknown how generalizable these findings are to the wider population of calves affected by septic arthritis in Norway and it would be valuable to examine a more representative population of cases that are presented to veterinary practitioners in the field. Further research could increase the timeframe for sampling and provide financial-support and incentivize sampling of cases by practicing veterinarians in order to increase submissions of

pre-treatment joint fluid samples from cases presenting in the field. This would help to identify whether the diagnostic methods, bacterial species involved, and prognosis for calves presenting with septic arthritis gents differ from those found in calves in Norway that were referred to a veterinary school.

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I would also like to thank veterinarian Albertine Namork for letting me join in her practice, and giving me the option to discuss common diagnostic and treatment approaches, which informed my "how to do" and veterinary submission form.

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Finally, thanks to my sister, Katarina Wara Sederholm for her expertise in Excel that helped me produce the figures presented here.

Summary

Title: Forekomst av bakterier hos kalver med leddbetennelse i Norge

Authors: Silje Sederholm

Lead Supervisor: Clare Phythian, Institusjon for Produksjonsmedisin, Sandnes

Co-supervisor: Adam Martin, Institusjon for Produksjonsmedisin, Oslo

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Appendices

Appendix 1: Example of the structure of the clinical and pathological database (Excel file)

Appendix 2: Copy of Facebook recruitment strategy and public responses

Appendix 3: Instruction on taking a joint sample («Instruks Hvordan ta leddprøve»)

Appendix 4: Veterinary form for submitting joint sample («Følgeskjema for leddprøve uttak»)

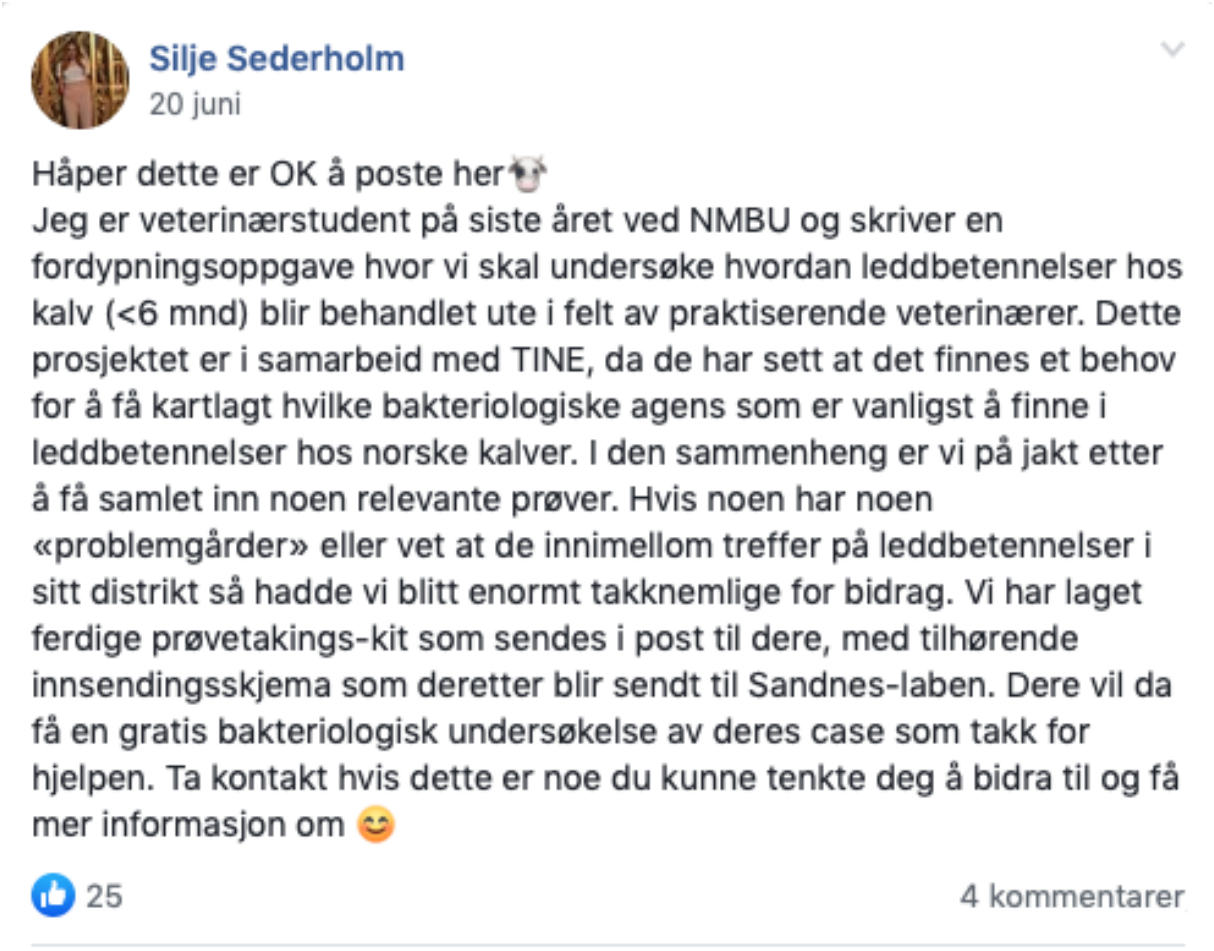
Appendix 1

Example of data management in Excel

(Orange - journal number, yellow – age (days), green - affected joint, blue – arthrocentesis results available and purple – bacteriology findings)

	A	B	E	F	G
1	JOURNAL- NR	ALDER	LEDD AFFISERT	DYRKET FOR BAKTERIER	VEKST AV BAKTERIER FUNNET
2	2018-856	60	Polyartritt	Ja	Enterococcus sp
3	2018-393	28	Polyartritt	Ja	Ingen funn
4	2018-401	<180	Klauvledd	Ja	Trueperella pyogenes, Pasterurella Sp
5	2018-396	10	Polyartritt	Ja	Escherichia Coli
6	2018-394	45	Carpalledd	Nei	0
7	2018-391	23	Polyartritt	Nei	0
8	2018-379	18	Polyartritt	Ja	Ingen funn
9	2018-372	43	Carpalledd	Ja	Ingen funn
10	2018-373	10	Polyartritt	Ja	Escherichia Coli
11	2018-304	22	Polyartritt	Nei	0
12	2018-265	34	Polyartritt	Ja	Trueperella pyogenes
13	2018-261	43	Polyartritt	Ja	Staphylococcus sp, Escherichia Coli
14	2018-241	41	Carpalledd	Nei	0
15	2018-218	37	Carpalledd	Nei	0
16	2018-107	66	Bakkneledd	Ja	Ingen funn
17	2018-134	35	Polyartritt	Nei	0
18	2017-944	103	Polyartritt	Ja	Ingen funn
19	2017-826	118	Hofteledd	Ja	Escherichia Coli + Trueperella Pyogenes + Cl.perfereinges + Enterococcus sp
20	2017-794	46	polyartritt	Ja	Enterococcus + Staphylococcus Sp

Appendix 2



Silje Sederholm
20 juni

Håper dette er OK å poste her🐮
Jeg er veterinærstudent på siste året ved NMBU og skriver en fordypningsoppgave hvor vi skal undersøke hvordan leddbetennelser hos kalv (<6 mnd) blir behandlet ute i felt av praktiserende veterinærer. Dette prosjektet er i samarbeid med TINE, da de har sett at det finnes et behov for å få kartlagt hvilke bakteriologiske agens som er vanligst å finne i leddbetennelser hos norske kalver. I den sammenheng er vi på jakt etter å få samlet inn noen relevante prøver. Hvis noen har noen «problemgårder» eller vet at de innimellom treffer på leddbetennelser i sitt distrikt så hadde vi blitt enormt takknemlige for bidrag. Vi har laget ferdige prøvetakings-kit som sendes i post til dere, med tilhørende innsendingsskjema som deretter blir sendt til Sandnes-laben. Dere vil da få en gratis bakteriologisk undersøkelse av deres case som takk for hjelpen. Ta kontakt hvis dette er noe du kunne tenkte deg å bidra til og få mer informasjon om 😊

25 4 kommentarer

Appendix 3

Skjemaet postlegges sammen med prøver- Husk å merke prøvene hvis flere sendes sammen

Prøvetaking av leddbetennelse hos norsk kalv

Dato prøvetaking_____
Kalv ID_____
Veterinær_____

Produsentnummer_____
Bondes navn og telefonnummer_____
(Føres opp hvis bonde ønsker å bli kontaktet i forbindelse med studien. Eksempelvis oppfølgingsspørsmål om sykdomsforløpet til kalven)

Rase

☐ NRF ☐ Limousine ☐ Annet; spesifiser ☐ Vet ikke
☐ Hereford ☐ Jersey ☐ Blanding; spesifiser
☐ Charolais ☐ Angus

Kjønn

☐ Kvigekalv ☐ Oksekalv

Alder (< 6 mnd)
Fødselsdato (dd/mm/åååå) _____

Affisert ledd (fra hvor prøven er tatt)

☐ Carpus/forkneledd ☐ Tarsus/haseledd ☐ Bakkneledd

Flere ledd affisert? (Polyartritt)

☐ Ja ☐ Nei

Antibiotika behandling

☐ Benzylpenicillin ☐ Trimetoprim+sulfa ☐ Gentamicin

Antall dager behandlet _____ Dose brukt (mg/kg) _____

☐ Bruker ikke antibiotika

Smertestillende behandling

☐ Meloxicam ☐ Carprofen ☐ Ketoprofen ☐ Annet; spesifier_____
Antall dager behandlet _____ Dose brukt (mg/kg) _____

Spyling av ledd

☐ Ja ☐ Nei

OBS: På grunn av ferieavvikling vil ikke laboratoriet være bemannet en liten periode. Prøvene må ikke postlegges i perioden 21 juli – 12 august. De kan fint oppbevares i kjøleskap. Tusen takk for hjelpen ☺

Appendix 4

NB!
HUSK
RENE
(STERILE)
HANSKER!

Instruks

«Hvordan ta leddprøve»

Utstyr: Klippemaskin, såpe, desinfeksjonsmiddel, kanyle (16 eller 18 G), kullsvaber

Alltid start med klipp, vask & desinfeksjon. Ved klipping er det viktig å bøye leddet etter å ha klippet da huden kan flytte på seg. Huden må være godt vasket og desinfisert før prøvetaking siden det skal foregå en injeksjon inn i ledd som har et sterilt miljø.
Det er også viktig med vask og desinfeksjon av huden etter prøvetaking.

***Forkne/carpus*:**
Radiocarpale og mediocarpale leddet: Nål stikkes rett inn (90 grader) på det dorsolaterale aspektet mellom de laterale collaterale ligamenter og *musculus radialis extensor*, ved bøying av kneet

***Bakkne/stifle*:**
Femoropatellare leddet: Nål stikkes rett inn (90 grader) mellom de mediale og midtre patellaligamenter, 3 cm proximalt for *tibialis tuberositas.*
Femorotibiale leddet: Nål stikkes rett inn (90 grader) i den laterale posen av den craniale eller caudale grensen av *musculus longus digitalis extensor*, mellom *tibialis tuberositas* og den laterale kondylen

***Has/hock/tarsus*:**
Tarsocrurale leddet: Nål føres inn mellom det laterale collaterale ligamentet og senen til tilheftningspunktet av *musculus tibialis cranialis*

Forkne *Bakkne* *Has*