Veterinary Immunology and Immunopathology 183 (2017) 22–30

Contents lists available at ScienceDirect

Veterinary Immunology and Immunopathology

journal homepage: www.elsevier.com/locate/vetimm

Research paper

Transfer of maternal immunity to piglets is involved in early protection against *Mycoplasma hyosynoviae* infection

K. Tølbøll Lauritsen[a,b,*], T. Hagedorn-Olsen[a,1], G. Jungersen[a], U. Riber[a], H. Stryhn[c], N.F. Friis[a,3], P. Lind[a], B. Kristensen[b,2]

[a] National Veterinary Institute, Technical University of Denmark, Bülowsvej 27, 1870 Frederiksberg C, Denmark
[b] Department of Disease Biology, Faculty of Health and Medical Sciences, University of Copenhagen, Stigbøjlen 4, 1870 Frederiksberg C, Denmark
[c] Department of Health Management, University of PEI, Charlottetown, Prince Edward Island C1A 4P3, Canada

ARTICLE INFO

Article history:
Received 5 June 2016
Received in revised form
12 November 2016
Accepted 3 December 2016

Keywords:
Mycoplasma hyosynoviae
Arthritis
Colostrum
Antibody
Pig
Lymphocyte proliferation

ABSTRACT

Mycoplasma hyosynoviae causes arthritis in pigs older than 12 weeks. The role of colostrum in protection of piglets against *M. hyosynoviae* infection is not clear. Our objective was therefore to investigate whether transfer of maternal immunity to piglets was involved in early protection against the infection. Experimental infections were carried out in three groups of weaners receiving different levels of *M. hyosynoviae*-specific colostrum components; Group NC derived from Mycoplasma free sows and possessed no specific immunity to *M. hyosynoviae*. Group CAb pigs, siblings of the NC group, received colostrum with *M. hyosynoviae*-specific antibodies immediately after birth. Group CCE pigs were born and raised by infected sows and presumably had the full set of colostrally transferred factors, including specific antibodies. When 4½ weeks old, all pigs were inoculated intranasally with *M. hyosynoviae*. The course of infection was measured through clinical observations of lameness, cultivation of *M. hyosynoviae* from tonsils, blood and synovial fluid and observation for gross pathological lesions in selected joints. Specific immune status in the pigs was evaluated through detection of antibodies by immunoblotting and measurement of *M. hyosynoviae*-specific T-cell proliferation. The latter analysis may possibly indicate that *M. hyosynoviae* infection induces a T-cell response. The CCE piglets were significantly protected against development of lameness and pathology, as well as infection with *M. hyosynoviae* in tonsils, blood and joints, when compared to the two other groups. Raising the CCE pigs in an infected environment until weaning, with carrier sows as mothers, apparently made them resistant to *M. hyosynoviae*-arthritis when challenge-infected at 4½ weeks of age. More pigs in group NC had *M. hyosynoviae* related pathological lesions than in group CAb, a difference that was significant for cubital joints when analysed on joint type level. This finding indicates a partially protective effect of passively transferred *M. hyosynoviae*-specific colostral antibodies upon development of *M. hyosynoviae* related pathology. Thus, the level of passive immunity transferred from sow to piglet seems to provide, at least partial, protection against development of arthritis. It cannot be ruled out that the CCE pigs, by growing up in an infected environment, have had the chance to establish an active anti-*M. hyosynoviae* immune response that complements the maternally transferred immune factors. Evident from this study is that the general absence of *M. hyosynoviae* arthritis in piglets can be ascribed mainly to their immunological status.

1. Introduction

Mycoplasma hyosynoviae infection is a common cause of acute and severe lameness among Danish growing-finishing pigs (Nielsen et al., 2001). Herds with severely affected pigs experience increased use of antibiotics and workload as well as reduced animal welfare (Kobisch and Friis, 1996; Nielsen et al., 2001). The prevalence of *M. hyosynoviae* in the Danish swine industry has not been investigated thoroughly, however non-published experiences form Danish pig herds indicate that the majority of these are infected.

* Corresponding author at: Section for Diagnostic and Scientific Advice, National Veterinary Institute, Technical University of Denmark, Bülowsvej 27, 1870 Frederiksberg C, Denmark.
E-mail address: ktla@vet.dtu.dk (K.T. Lauritsen).
[1] Present address: Dako Denmark A/S, Produktionsvej 42, 2600 Glostrup, Denmark.
[2] Klinik for Veterinær Dermatologi, Skovalleen 31, 2880 Bagsværd, Denmark.
[3] Deceased. N.F. Friis approved the manuscript before he passed away.

http://dx.doi.org/10.1016/j.vetimm.2016.12.002
0165-2427/

M. hyosynoviae is harboured in the tonsils of infected pigs (Ross and Spear, 1973; Friis et al., 1991). This carrier state is primarily established in pigs above ten weeks of age and infection is rarely transmitted from sows to piglets (Hagedorn-Olsen et al., 1999a). Via the blood stream the mycoplasmas may spread to the joints (Kobisch and Friis, 1996; Hagedorn-Olsen et al., 1999b) and cause arthritis in pigs above 12 weeks of age (Ross and Duncan, 1970; Hagedorn-Olsen et al., 1999a). A previous experiment showed that 6-week-old pigs, immunologically naive with respect to *M. hyosynoviae*, were able to develop acute joint infection within 2–13 days after intranasal inoculation with the agent (Lauritsen et al., 2008). This indicated that the absence of *M. hyosynoviae* related lameness in this age group under field conditions must have another explanation than strictly age related factors.

Sow colostrum contains antibodies, which the newborn piglets absorb to the circulation through the gut (Frenyo et al., 1981; Klobasa et al., 1981; Rooke and Bland, 2002; Salmon et al., 2009; Bandrick et al., 2011; Nechvatalova et al., 2011), as well as other immunological components such as cells of the immune system, e.g. neutrophils and eosinophils, macrophages and lymphocytes (Evans et al., 1982; Schollenberger et al., 1986a,b; Magnusson et al., 1991, Nechvatalova et al., 2011). Live cells from sow colostrum are transferred across the gut epithelium of the piglet and into the blood/lymphatics (Tuboly et al., 1988; Williams, 1993; Salmon, 2000; Salmon et al., 2009; Nechvatalova et al., 2011) and it has been discussed in several papers whether colostral cells actively could comprise a pool of cellular immunocompetence that can be transferred from sow to the suckling piglet (Salmon, 2000; Wagstrom et al., 2000; Salmon et al., 2009; Nechvatalova et al., 2011). The role of colostrum in protection of piglets against *M. hyosynoviae* infection has so far not been clarified, although the abovementioned results by Lauritsen et al. (2008) may point in the direction of presence of maternally transferred immunity. Antibodies specific for *M. hyosynoviae*, presumably originating from colostrum, have been shown to be present in suckling piglets (Blowey, 1993; Hagedorn-Olsen et al., 1999a). In the present study the hypothesis was that transfer of maternal immunity to piglets is involved in early protection against *Mycoplasma hyosynoviae* infection. The possible role of specific *M. hyosynoviae* antibodies was investigated by developing an experimental colostrum model. One group of piglets were isolated from the sow immediately after birth and fed cell-free colostrum containing significant levels of specific *M. hyosynoviae* antibodies (Colostrum antibody group – CAb group). Protection of this group after inoculation with *M. hyosynoviae* was compared to two other groups, one that had suckled infected sows (Complete Colostrum and Exposure group – CCE group) and one that had suckled sows that were immunologically naive to *M. hyosynoviae* (Naive Colostrum group – NC group).

2. Materials and methods

2.1. Animal material and housing conditions

Thirty-two pigs were allocated to three groups, subjected to different regimens of colostrum intake; (i) the CAb group received *M. hyosynoviae*-specific antibodies via colostrum that had been frozen and thawed to destroy live cells, (ii) the NC group suckled colostrum without *M. hyosynoviae*-specific immunity and was therefore immunologically naive, (iii) the CCE group received complete colostrum containing antibodies and cellular components from their *M. hyosynoviae* infected mothers, and was exposed to infected environment until weaning. The experimental design is illustrated in Fig. 1. All pigs included in the study were crossbreds (offspring from Danish Landrace/Yorkshire sows and Duroc or Hampshire boars). The CAb and NC groups were kept isolated under experimental conditions from birth, whereas the CCE group was transferred to the experimental facilities one week before inoculation (Fig. 1). All pigs of the study were weaned at 3–3½ weeks of age (Fig. 1). The pigs were kept loose in pens with concrete floors and abundant straw-bedding. Fresh water was supplied *ad libitum* through water nipples, and the pigs were fed factory-made pelleted standard swine feed without addition of any antimicrobials.

2.2. Preparation of the colostrum pool

The colostrum artificially fed to the CAb group (Fig. 1) was prepared from colostrum of four sows from a *M. hyosynoviae* infected herd, but not the same herd which supplied pigs for the CCE group. Within 24 h after parturition 300–600 ml of colostrum was collected from each sow using the following method; Sows were prepared for colostrum collection by i.v. injection of 20 IU oxytocin (Oxytocin®, Leo Vet) and the udder was washed and disinfected (0.5% chlorhexidin in 70% ethanol). Colostrum was collected by hand stripping into sterile wide mouth glass bottles. After removing 2–4 ml for later cultivation for *M. hyosynoviae*, 200 mg tiamulin (Tiamutin ®, Novartis) was added per 100 ml colostrum and the colostrum was stored at −20 °C. Further, the colostrum was thawed, pooled, filtered through sterile gauze, aliquoted into sterile bottles and stored at −20 °C until use. Before being fed to the newborn pigs, the colostrum was thawed in a lukewarm water bath, and the temperature adjusted to 38 °C. Further details on the colostrum feeding to piglets are described in Fig. 1. All colostra used were cultivation negative for *Mycoplasma spp* prior to addition of Tiamulin.

2.3. Inoculation with M. hyosynoviae

Between 4 and 4½ weeks of age all pigs were inoculated with a cloned field strain of *M. hyosynoviae*, Mp927 (titres 10^7–10^8 colour changing units (CCU) per ml). The method used for preparation of inoculum is described by Lauritsen et al. (2008). Pigs were inoculated intranasally into the *dorsal meatus*, while in dorsal recumbency. Inoculation dose was 1 ml in each nostril.

2.4. Mycoplasma cultivation

Cultivation for *M. hyosynoviae* from heparin-stabilized blood samples was performed on post inoculation day (PID) 4, 7, 9, 12 and 15. Tonsillar scrapings, obtained with a sterile blunt steel scraper especially designed for the purpose, were collected two days before inoculation and on PID 4, 7, 9 and 12. Cultivation for *M. hyosynoviae* in colostrum was performed in 1:10 serial dilutions to 10^{-4} in modified Hayflick's medium (Kobisch and Friis, 1996). All other methods, used in this study for mycoplasma cultivation, including production of inoculation material, have been described by Lauritsen et al. (2008).

2.5. Clinical recordings and post mortem examinations

Prior to inoculation all pigs had a normal body condition and did not show any clinical signs of disease. Every day post inoculation, the pigs were observed for clinical signs of lameness and other signs of disease. The pigs were euthanized and autopsied on PID 12, 14 or 16, i.e. in the time period of expected occurrence of the acute infection phase (Kobisch and Friis, 1996; Hagedorn-Olsen et al., 1999c). The date of euthanasia for each pig was determined before inoculation and pigs from each group were evenly represented on the necropsy days. Euthanasia was performed by stunning with a captive bolt pistol followed by exsanguination. At autopsy, six joints per pig were examined for gross pathological lesions since we focused on cubital, stifle and tibiotarsal joints. For each joint the