

## Absence of *Campylobacter* in some Norwegian Specific Pathogen Free (SPF) Pig Herds

Evan Mark Kolstoe<sup>1</sup>, Terje Iversen<sup>2</sup>, Øyvind Østensvik<sup>1</sup>, Truls Nesbakken<sup>1\*</sup>

<sup>1</sup> Norwegian School of Veterinary Science, Department of Food Safety and Infection Biology, P.O. Box 8146 Dep., 0033 Oslo, Norway

<sup>2</sup> Nortura BA, P.O. Box 2508, 7729 Steinkjer, Norway

\*corresponding author: [truls.nesbakken@veths.no](mailto:truls.nesbakken@veths.no)

### Abstract

In this study, ten Norwegian Specific Pathogen Free (SPF) pig herds (three nucleus and seven multiplying herds) were surveyed for *Campylobacter* spp. to determine the occurrence of this organism in the Norwegian SPF pig health and breeding pyramid. From the ten SPF herds, four tested negative for *Campylobacter* spp., one showed presence of an unknown *Campylobacter* spp. and the remaining five herds tested positive for *C. coli*. Of the three nucleus herds which were tested, only the first established nucleus herd was free from *C. coli*. The three production herds testing negative for *Campylobacter* were all established with gilts from the first nucleus herd. The herds testing positive for *Campylobacter* were all established with animals from either one or both of the positive nucleus herds. Three of the five herds that were carriers of *C. coli* had a prevalence rate of 37.5% while the lowest rate was in nucleus herd 2 with 12.5%. The remaining herd showed a prevalence of 69.2%.

### Introduction

*Campylobacter* is one of the most common agents of acute human gastroenteritis. *Campylobacter jejuni* and *Campylobacter coli* can all be recovered from the intestinal tracts of many animals leading to the contamination of foods from animal origins. Multiple studies have shown that pigs are an important reservoir of *C. coli*, along with other *Campylobacter* spp. and that the prevalence of carriage in pigs is widespread throughout the world (Humphrey et al., 2007; Young et al., 2000; Weijtens et al., 1997). In other countries Specific Pathogen Free (SPF) herds have been established under varying levels of hygienic controls and some of them have been able to remain free from *Campylobacter* (Leblanc Maridor et al., 2008; Weijtens et al., 2000).

In the current Norwegian pig production system, the majority of pigs still come from the traditional breeding system where most herds are carriers of *Campylobacter coli* (Rosef et al., 1983). However, there has been an increase in the establishment of new SPF herds these last years in Norway (Helsetjenesten for svin, 2006). While SPF herds currently provide health management and economic benefits to the farmers, the benefits to the food safety system have not yet been fully utilized. Since Norwegian SPF herds are managed to prevent specific pig diseases, it might be feasible to expand the list of microorganisms even further to include human pathogens and thus create Norwegian Specific Human Pathogen Free (SHPF) production pig herds which would benefit the food safety system.

In this study, ten SPF pig herds (three nucleus and seven multiplying herds) were surveyed for *Campylobacter* spp. to determine the prevalence of this organism in the Norwegian SPF pig health and breeding pyramid.

### Materials and methods

#### *The herds*

In 1997, the first Norwegian SPF nucleus herd which now has about 100 breeding sows (herd 1 in Table 1) was established by hysterectomy, and the piglets were reared without contact with other pigs. In 1999, a second nucleus SPF herd which now has about 65 breeding sows (herd 2 in Table 1) was established

with gilts from herd 1. These two herds have been totally isolated from other herds, except for artificial insemination. Since the formation of the first two nucleus herds, a third herd in 2005 classified as a nucleus herd was established with gilts from herd 2, and 30 production herds have been established with pigs from the nucleus herds. There are also an additional 20 herds which house only slaughter age pigs coming from the piglet producing SPF herds. All of these herds are classified as SPF herds. The nucleus SPF herds are housed, the water supply is potable, and pest control systems are established. Pets and wild animals cannot enter the pig house. The owner, herdsman, veterinarians, technicians, and all others visiting must shower and change clothes before entering the pig house. For this study ten SPF herds were selected based on farmer participation including all three of the nucleus herds.

#### *Collection of fecal samples*

Each herd was sampled once. In total 399 samples were collected. From each herd visited ten samples each were collected from piglets and sows, while twenty samples were collected from slaughter age pigs for a total of 40 samples collected at each farm. However, in herd number one, 19 instead of 20 samples were collected from the slaughter age pigs and 9 of those samples were from gilts.

Fecal samples tested weighed between 1.0-10 g. The average amounts tested per herd varied from an average of 7.9 g (range 1.0-10.0 g) to an average of 9.7 g (range 6.5-10.0 g). The fecal samples were aseptically collected from the rectum of the pigs by use of a clean plastic glove, sealed, put into a box and held at 4 °C. The samples were transported to the laboratory where analysis was initiated within 24 h after collection.

#### *Isolation and characterization of Campylobacter spp.*

*Campylobacter* was cultured and isolated following the Nordic Committee on Food Analysis method No. 119 3rd ed. (2007) (qualitative detection) with modifications as described below. All medias used were Oxoid Ltd. (Basingstoke, Hampshire, England) unless otherwise stated, and microaerobic atmospheres were created using anaerobic jars with CampyGen™ CN 25 sachets (Oxoid).

An amount of 10 g of sample was transferred into a BagFilter stomacher bag (Interscience, Saint Nom La Bretèche, France) along with 90 g buffered peptone water (BPW) and stomached in an IUL Masticator (IUL sa, Barcelona, Spain). In cases where less than 10 g of sample was collected, the total amount collected was used and brought up to a 1:10 dilution in BPW. 1 ml of the dilution was transferred into 9 ml Preston broth and incubated at  $42 \pm 1.0$  °C for  $48 \pm 4$  h in a microaerobic atmosphere. A loopful, ~5 µl, of broth taken from under the surface was transferred to a mCCDA plate and incubated at  $42 \pm 1.0$  °C for  $48 \pm 4$  h in a microaerobic atmosphere. Two *Campylobacter* like colonies were then subcultured to mCCDA again to ensure pure colonies. One typical colony was then subcultured onto two blood agar plates. One inoculated plate was incubated in a microaerobic atmosphere at  $42 \pm 1.0$  °C for  $24 \pm 3$  h while the other was incubated in an aerobic atmosphere at  $42 \pm 1.0$  °C for  $24 \pm 3$  h to exclude bacteria which grow under aerobic conditions. *Campylobacter* conformation and further classification to species was carried out by using a microscope with dark field contrast for identifying morphology and motility, and testing for oxidase (Remel Inc., Lenexa, KS, USA), hydrolysis of indoxyl acetate (Remel), and hydrolysis of sodium hippurate (Becton, Dickinson & Co., Sparks, MD, USA) reactions.

## **Results**

From the ten SPF herds tested, four herds tested negative for *Campylobacter* spp., one showed presence of an unknown *Campylobacter* spp. and the remaining five herds tested positive for *C. coli* (Table 1). Of the three nucleus herds which were tested only the first established nucleus herd was free from *C. coli*. The three multiplying herds (herds 4, 5 and 10) which tested negative for *Campylobacter* were all established with gilts from herd number 1. In herd 7, which was also established from herd number 1, the samples tested positive for oxidase and catalase along with showing the correct morphology and motility under the microscope and had no aerobic growth. However they tested negative for hydrolysis of indoxyl acetate and sodium hippurate. They were also subcultured at 25°C without showing any growth. Diagnosis was

then stopped on the samples from herd 7. The herds which tested positive for *C. coli* were all established with animals from either one or both of the positive nucleus herds. Three of the five herds that were carriers of *C. coli* had a prevalence rate of 37.5% while the lowest rate was in nucleus herd 2 at 12.5%. The remaining herd showed a prevalence of 69.2%. Herd sizes ranged from 50 to 170 animals.

Table 1. Occurrence of *Campylobacter* spp. in ten SPF herds

Herd no. (year established) n = number of breeding sows	Number of carriers with <i>Campylobacter</i> spp. (%) based on investigation of				<i>Campylobacter</i> species diagnosis
	Piglets (n=10)	Sows (n=10)	Slaughter pigs <sup>c</sup> (n=20 <sup>a</sup> )	Total (n=40 <sup>b</sup> )	
<b>Nucleus herds</b>					
1 (1996) n=100	0	0	0	0	
2 (1999) n=65	1 (10)	1 (10)	3 (15)	5 (12.5)	<i>Campylobacter coli</i>
3 (2005) n=80	7 (70)	5 (50)	15 (78.9) <sup>ac</sup>	27 (69.2) <sup>b</sup>	<i>Campylobacter coli</i>
<b>Multiplying herds</b>					
4 (1998) n=50	0	0	0	0	
5 (2001) n=60	0	0	0	0	
6 (2003) n=170	2 (20)	1 (10)	12 (60)	15 (37.5)	<i>Campylobacter coli</i>
7 (2004) n=50	10 (100)	3 (30)	17 (85)	30 (75)	(not diagnosed)
8 (2006) n=60	2 (20)	10 (100)	3 (15)	15 (37.5)	<i>Campylobacter coli</i>
9 (2001) n=150	1 (10)	8 (80)	6 (30)	15 (37.5)	<i>Campylobacter coli</i>
10 (2007) n=56	0	0	0	0	

<sup>a</sup> The number of slaughter pigs tested in herd 3 is 19

<sup>b</sup> The total number of pigs tested in herd 3 was 39

<sup>c</sup> 9 of the pigs tested in herd 3 were gilts

## Discussion

This study has shown that sections of the current Norwegian SPF pig production system might be free from *Campylobacter*. Of the ten herds tested, four showed no evidence of carrying *Campylobacter* which is consistent with the results from previous research on other SPF farms considered to be *Campylobacter* free (Leblanc Maridor et al., 2008; Weijtens et al., 2000). However, as this is the first survey conducted of *Campylobacter* prevalence in Norwegian SPF pig herds, there is no historical data available for comparison.

According to the farmers, most of the water sources found on the relevant farms are supplied by the municipal water system. Since the water is treated, and it is the same water that the surrounding inhabitants drink, it is doubtful that this is an important vehicle of transmission in this case. While water is a common source of human infections in Norway, it is not the only possible route of transmission. A study in the Netherlands found no traces of *Campylobacter* in the water or feed on two Dutch poultry farms with their results pointing towards horizontal transmissions from the environment (Jacobs-Reitsma et al., 1995). Weijtens et al. (2000) also suggest that *Campylobacter* would be more likely introduced by the farmer himself (boots, hands, etc.) or by vermin (rodents, birds, flies, etc). Weijtens et al. (2000) go on to suggest that introduction by farmers might explain limited infection rates, such as those seen on four of the farms in this study, since if water or feed was the common source, higher prevalence rates on those affected farms might be expected. This does not mean that feed or water can be ruled out completely though, as this could be a source of contamination while still possibly explaining the limited infection

rates if the feed or water was only sporadically contaminated. But, as no environmental samples were collected, vermin captured, or water tested, there is no evidence in this study to point towards a contamination source.

Other possible explanations for the presence of *Campylobacter* in SPF pig herds might be found in the history of the farms and buildings used to house the animals. SPF herds can either be established on locations or in buildings which have housed pigs before or not. Introducing animals where there have been pigs before, even after cleaning might increase the risk of reintroduction of bacteria into the herd due to carry over if the facility is not kept free long enough or cleaned sufficiently. Management practices on the farm could also be another explanation. The use of specific bedding materials such as straw has been shown to be a factor in the spreading of *Yersinia enterocolitica* on pig farms (Skjerve et al., 1998), and even the use of saw dust on the floor in the pig house can be important. In conclusion, everything that comes from outside the protected herd can possibly harbor unwanted bacteria unbeknownst to the farmer.

Since all of the herds testing negative for *Campylobacter* were established with animals from herd 1, this suggests that if the top of the breeding chain is kept free from *Campylobacter*, then any herds maintained under that herd in the breeding pyramid can remain free as well if properly protected.

SPF herds have many advantages for continued and further developments. These herds have been shown to have cost-benefits for farmers by lowering the costs of veterinary services and medicines as well as supplying animals which have a greater feed to weight ratio (Hofmo and Narum, 2004). In the future, SPF herds can benefit food safety by becoming SHPF herds with a goal of one day ultimately being classified as Human Pathogen Free (HPF) herds. To be classified as HPF, herds must be free from all major human pathogens of which *Campylobacter* is included. By being classified as SHPF or HPF herds, this can bring a premium price for pork products helping in the cost analysis factor while making the supply chain safer for consumers. However, since *Campylobacter* is already controlled under current Norwegian processing techniques such as blast chilling (Nesbakken, et al. 2008), attention should be focused on establishing SHPF instead of HPF pig herds. As per today, SPF herds are already probably mostly free from *Salmonella*, *Trichinella*, *Toxoplasma gondii*, and *Y. enterocolitica* (Nesbakken et al., 2007). Therefore, the establishment of Norwegian SHPF pig herds would be possible if the list is defined as free from *Salmonella*, *Trichinella*, *T. gondii*, and *Y. enterocolitica*.

## Conclusion

In conclusion, this study has shown that under the current structure and management practices of commercial SPF herds in Norway, some herds are *Campylobacter* free while others are not. This would suggest that while some of the herds have sufficient hygiene barriers and practices already in place, the overall regulation of the entire SPF production chain is not yet fully enough developed to provide *Campylobacter* free herds, and the introduction of a full HPF breeding chain still needs much development in Norway.

## References

- Helsetjenesten for svin, 2006. Langtidsplan for Helsetjenesten for svin 2006-2010. Veterinærinstituttet, Oslo, Norway.
- Hofmo, P.O., Narum, M., 2004. SPF - framtidens norske gris? *Praksisnytt* 9 (4), 47-52.
- Humphrey, T., O'Brien, S., Madsen, M., 2007. *Campylobacters* as zoonotic pathogens: a food production perspective. *International Journal of Food Microbiology* 117, 237-257.
- Jacobs-Reitsma, W.F., Van de Giessen, A.W., Bolder, N.M., Mulder, R.W.A.W., 1995. Epidemiology of *Campylobacter* spp. at two Dutch broiler farms. *Epidemiology and Infection* 114, 413-421.
- Leblanc Maridor, M., Denis, M., Lalande, F., Beaurepaire, B., Cariolet, R., Fravalo, P., Federighi, M., Seegers, H., Belloc, C., 2008. Experimental infection of specific pathogen-free pigs with *Campylobacter*: excretion in faeces and transmission to non-inoculated pigs. *Veterinary Microbiology* 131, 309-317.

- Nesbakken, T., Eckner, K., Røtterud, O.J., 2008. The effect of blast chilling on occurrence of human pathogenic *Yersinia enterocolitica* compared to *Campylobacter* spp. and numbers of hygienic indicators on pig carcasses. *International Journal of Food Microbiology* 123, 130-133.
- Nesbakken, T., Iversen, T., Lium, B., 2007. Pig herds free from human pathogenic *Yersinia enterocolitica*. *Emerging Infectious Diseases* 13, 1860-1864.
- Nordic Committee on Food Analysis, 2007. Thermotolerant *Campylobacter*. Detection, semi-quantitative and quantitative determination in foods and drinking water. No.119 3. Ed.
- Rosef, O., Gondrosen, B., Kapperud, G., Underdal, B., 1983. Isolation and characterization of *Campylobacter jejuni* and *Campylobacter coli* from domestic and wild mammals in Norway. *Applied and Environmental Microbiology* 46, 855-859.
- Skjerve, E., Lium, B., Nielsen, B., Nesbakken, T., 1998. Control of *Yersinia enterocolitica* in pigs at herd level. *International Journal of Food Microbiology* 45, 195-203.
- Weijtens, M.J.B.M., Urlings, H.A.P., van der Plas, J., 2000. Establishing a *Campylobacter*-free pig population through a top-down approach. *Letters in Applied Microbiology* 30, 479-484.
- Weijtens, M.J.B.M., van der Plas, J., Bijker, P.G.H., Urlings, H.A.P., Koster, D., van Logtestijn, J.G., Huis in't Veld, J.H.J., 1997. The transmission of *Campylobacter* in piggeries; an epidemiological study. *Journal of Applied Microbiology* 83, 693-698.
- Young, C.R., Harvey, R., Anderson, R., Nisbet, D., Stanker, L.H., 2000. Enteric colonisation following natural exposure to *Campylobacter* in pigs. *Research in Veterinary Science* 68, 75-78.