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***Campylobacter* spp.: Risk factor analysis in fattening pig farms**

Abstract

There is a lack of information about the prevalence and origins of the important zoonotic pathogen *Campylobacter* spp. in the different stages of the pig production chain. The aim of this study was to gather further information about the sources of infection with *Campylobacter* spp. and their qualitative and quantitative importance in pig production. For statistical analysis, 1,040 results from the bacteriological examination for *Campylobacter* spp. were evaluated with questionnaires from four farrowing and twelve fattening units. The prevalence was determined via faeces and swab samples with regard to certain farm production parameters. Thereby 30.8% of the sows and 80.9% of their piglets were carriers of *Campylobacter* spp.. In the fattening unit, the prevalence at the beginning of the fattening period was 89.2% and at the end 64.7%. As a result of the small sample size in the farrowing unit it was not possible to perform a risk analysis which yielded significant conclusions. In the fattening stage, the following risk factors had a significant effect ($p \leq 0.05$) on *Campylobacter* spp. prevalence: sampling time, number of fattening places per herd, mixed farming, floor space design, feed origin, antibacterial and anthelmintic treatment. These results show that housing and management have a possible influence on the *Campylobacter* spp. prevalence and should be investigated further.

Key Words: *Campylobacter coli* / *jejuni*, pig, fattening units, risk analysis, odds ratio

Zusammenfassung

Titel der Arbeit: *Campylobacter* spp.: Risikoanalyse in Schweinemastbetrieben

Über die Prävalenzen und Eintragsquellen des Zoonoserregers *Campylobacter* spp. in den verschiedenen Produktionsstufen der Schweineerzeugung existieren bisher nur wenige Informationen. Die vorliegende Studie soll zur Aufdeckung produktionsspezifischer Risikofaktoren und ihrer Analyse hinsichtlich der qualitativen und quantitativen Bedeutung beitragen. Für die statistische Analyse wurden 1.040 Ergebnisse der bakteriologischen Untersuchung auf *Campylobacter* spp. im Zusammenhang mit den Informationen aus einem Fragebogen aus vier Ferkelerzeuger- und zwölf Mastbetrieben ausgewertet. Die Prävalenzen des Erregers wurden mit Hilfe von Kot- und Abstrichupferproben vor dem Hintergrund verschiedener Betriebsbedingungen ermittelt. Dabei wurden bei 33,8% der Sauen und bei 80,9% der Ferkel *Campylobacter* spp. nachgewiesen. In der Produktionsstufe Mast betrug die Prävalenz am Mastanfang 89,2% und am Mastende 64,7%. Aufgrund des geringen Datenmaterials konnte auf der Produktionsstufe Ferkelerzeugung keine Risikoanalyse durchgeführt werden. Folgende Faktoren hatten auf den Mastbetrieben einen signifikanten Einfluss ($p \leq 0,05$) auf die *Campylobacter* Prävalenz: Zeitpunkt der Probeentnahme, Anzahl Mastplätze, Mischbetrieb, Bodengestaltung, Futterherkunft, Einstallbehandlung und anthelminthische Behandlung. Die Ergebnisse veranschaulichen, dass eine Reduzierung der *Campylobacter* spp. Prävalenz durch betriebliche Haltungs- und Managementfaktoren möglich ist. Dieses Phänomen sollte weiter untersucht werden.

Schlüsselwörter: *Campylobacter coli* / *jejuni*, Schwein, Mastbetriebe, Risikoanalyse, Odds Ratio

Introduction

Infections caused by *Campylobacter* spp. (*C.*) are prevalent worldwide. *Campylobacter jejuni* and *C. coli* are by far the most common *Campylobacter* species infecting humans. Both species are associated with clinically indistinguishable diarrhoea in humans (NACHAMKIN, 2003). In Germany, the Robert-Koch-Institute

registered 61,823 cases of humans suffering from such an infection in 2005. However, *C. jejuni* is implicated in about 85% of the cases of human campylobacteriosis, with the remaining cases being primarily caused by *C. coli* (FRIEDMAN et al., 2000).

Campylobacter spp. are part of the normal gut microflora in many food-producing animal species, including chickens, turkeys, swine, cattle and sheep (BLASER, 1997). For instance, *C. jejuni* is more commonly isolated from chickens and cattle, while *C. coli* is more common among swine (YOUNG et al., 2000). Transmission to humans appears to occur primarily through the consumption of contaminated poultry products, unpasteurised milk products and meat products (EFFLER et al., 2001; FRIEDMAN et al., 2004). In addition to the consumption of undercooked meat, cross-contamination to other food products may play a significant role in the number of illnesses observed. The infective dose (number of organisms sufficient to cause infection) in humans can be very low. Only 800 colony-forming units of specific strains can lead to Campylobacter infection (BLACK, 1988).

According to the regulations of the “White Paper on Food Safety” (EUROPÄISCHES WEISSBUCH ZUR LEBENSMITTELSICHERHEIT, 2000), the farmer and the participating manufacturing industry in the food production should have the main responsibility for food safety. Now and in future, this adds up to the demand for preventive measures in primary production following the principle “from the producer to the consumer”. This leads to a consolidated need for the detection of relations between pathogen prevalence in the herds and the herd management and husbandry. Determination of various important entry routes and spreading factors provides useful decision guidance for all production units in the meat production chain to minimise the transmission of zoonotic pathogens. For these reasons, this study was conducted with the aim to determine the prevalence of *Campylobacter* spp. in farrowing and fattening units by the collection of faeces and rectal swabs. Further risk factors for the occurrence of *Campylobacter* spp. in farrowing and fattening units should be observed via environmental and feed samples from the checked herds and questionnaires in the corresponding pig farms.

Material and Methods

Four farrowing and twelve fattening farms provided the basis for the present study. The sampling size on every farm was calculated according to the formula from NOORDHUIZEN et al. (1997). In total, 1,040 faecal or swab samples respectively from pigs of all ages from farrowing and fattening units were analysed. Additionally, 56 environmental and feed samples were collected.

Cultural methods were used to test all samples for *Campylobacter* spp., including the differentiation of subspecies. The bacterial detection of *Campylobacter* spp. proceeds from ISO 10272 (1995) with following biochemical differentiation of *C. coli* and *C. jejuni*.

Calculation of the intraherd and animal prevalence and the 95%-confidence intervals within the production stage was performed with the PROC SURVEYMEANS procedure from SAS[®] (2002).

On every farrowing and fattening farm, data collection was carried out with the aid of a questionnaire. Besides the general farm information, detailed data about the housing system, management, state of health and aspects of disease surveillance were acquired.

In consideration of the bacteriological results, these data contributed to a hazard analysis to detect the origin and spread of *Campylobacter* spp. infections.

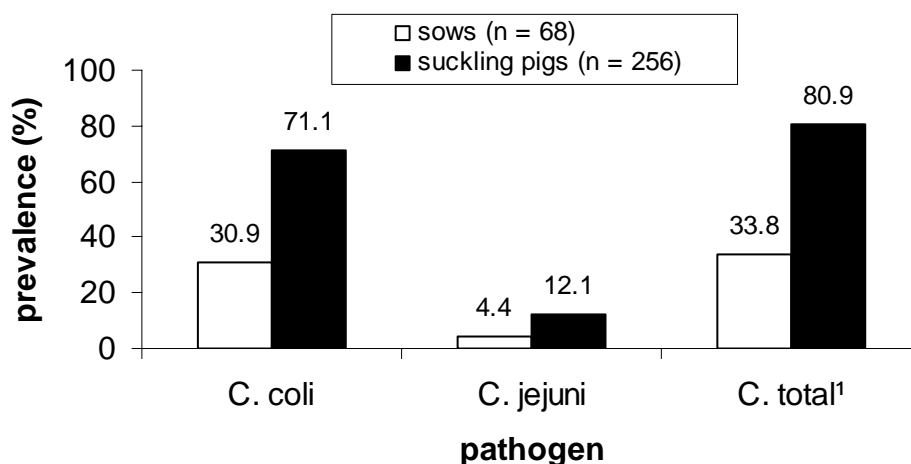
The statistical analysis was performed with a generalised linear model. At first the management-specific parameters were tested respectively with the χ^2 -test regarding the influence on the pathogen prevalence. Every parameter having a value $p < 0.3$ in the χ^2 -test and an adequate distribution was included in the generalised linear model. The GENMOD procedure from the software package SAS[®] (2002) was reviewed for significance ($p \leq 0.05$). For the estimation, a binomial distribution and a logistic link function (i.e. logistic regression) were assumed. As a result of the small sample size in the farrowing unit, it was not possible to perform a risk analysis which yielded significant conclusions. From the fattening unit, the following fixed effects were considered in the model: sampling time (growing pigs, finishing pigs), herd organisation (number of fattening places, mixed farming), housing system and forage (floor space design, feed origin) and health (antibacterial and anthelmintic treatment). The estimates ($\hat{\epsilon}$) from the risk factors were transformed into odds ratios ($OR = \exp(\hat{\epsilon})$) and the 95%-confidence intervals were calculated. A low absolute frequency in the least sub classes from some factors did not allow a statistical analysis with logistic regression. For the factors having a p-value ≤ 0.05 in the χ^2 -test, the odds ratios and 95%-confidence intervals were calculated separately.

Results

Prevalence

Sows and suckling pigs

Campylobacter (*C.*) spp. were isolated in 33.8% of the sows and in 80.9% of the piglets (Figure 1). Neither pathogen was isolated from the environmental and feed samples.



¹ *C. total* = *C. coli* and/or *C. jejuni*

Fig. 1: Prevalence of *Campylobacter* spp. in sows and suckling pigs (Prävalenz von *Campylobacter* spp. bei Sauen und Saugferkeln)

Table 1 shows the prevalence of *Campylobacter* spp. in pigs of the farrowing unit at herd level. Notable is the fact that in herd 4 no sows are carriers of the pathogen but

some of their piglets are. In herd 3, no piglets were sampled, therefore no results for this production stage appear in Table 1.

Table 1

Prevalence of *Campylobacter* spp. in pigs of the farrowing unit at herd level (Prävalenz von *Campylobacter* spp. in der Ferkelerzeugung auf Betriebsebene)

		sows ¹		suckling pigs ²	
		prevalence (%)	95%-CI ³	prevalence (%)	95%-CI
herd 1	<i>C. coli</i>	23.5	1.1-46.0	96.5	92.5-100.0
	<i>C. jejuni</i>	-	-	-	-
	<i>C. total</i> ⁴	23.5	1.1-46.0	96.5	92.5-100.0
herd 2	<i>C. coli</i>	94.1	81.6-100.0	95.3	90.8-99.9
	<i>C. jejuni</i>	-	-	-	-
	<i>C. total</i>	81.6	81.6-100.0	95.3	90.8-99.9
herd 3	<i>C. coli</i>	5.9	0-18.4	-	-
	<i>C. jejuni</i>	17.6	0-37.9	-	-
	<i>C. total</i>	17.6	0-37.9	-	-
herd 4	<i>C. coli</i>	not sampled	not sampled	21.2	12.3-30.0
	<i>C. jejuni</i>	not sampled	not sampled	36.5	26.0-46.9
	<i>C. total</i>	not sampled	not sampled	50.6	39.7-61.4

¹n = 17 per herd

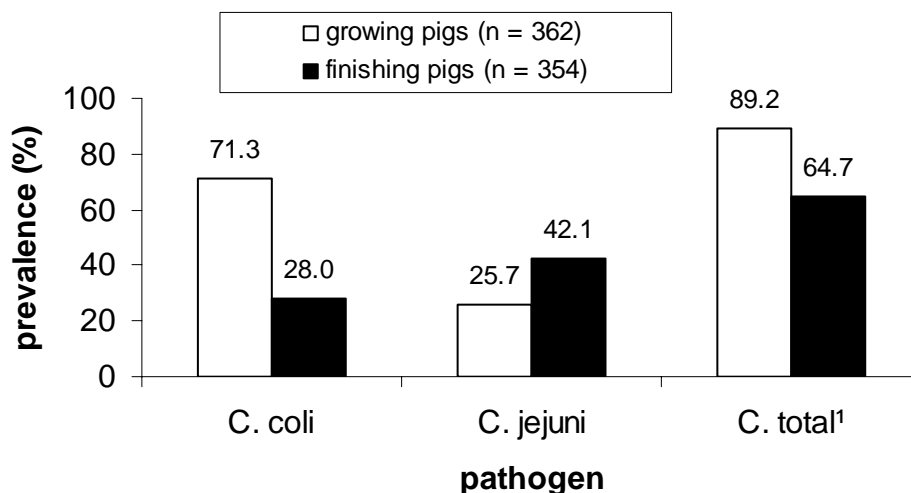
²n = 85 or 86 per herd

³95%-confidence interval

⁴*C. total* = *C. coli* and/or *C. jejuni*

Fattening pigs

The prevalence of *Campylobacter* spp. in growing pigs was 89.2% and in finishing pigs slightly lower with 64.7% (Figure 2). Neither pathogen was isolated from the environmental and feed samples.

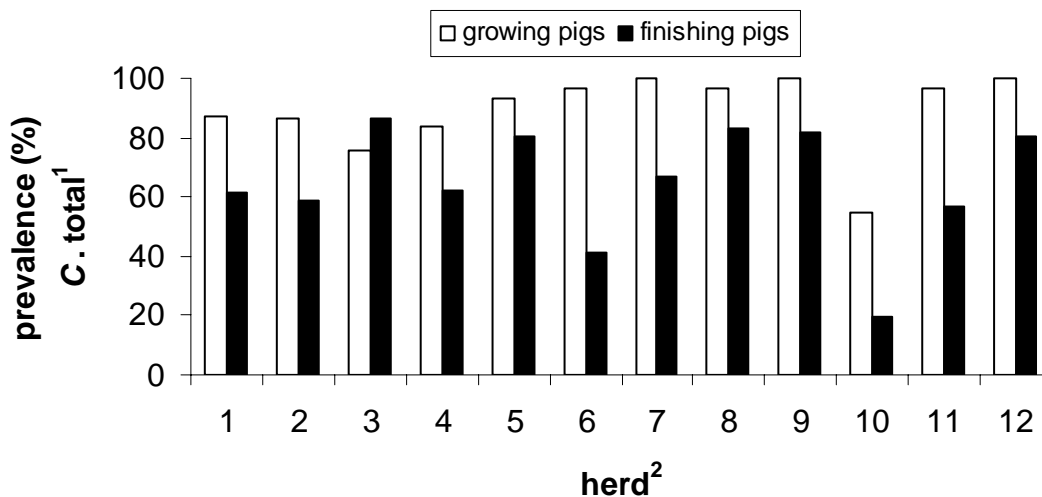


¹ *C. total* = *C. coli* and/or *C. jejuni*

Fig. 2: Prevalence of *Campylobacter* spp. in growing and finishing pigs (Prävalenz von *Campylobacter* spp. am Mastanfang bzw. Mastende)

Campylobacter spp. were detected on all farms in growing and finishing pigs (Figure 3). Herd 10 was the farm with the lowest *Campylobacter* spp. prevalence (54.8% in growing pigs and 19.4% in finishing pigs). In herd 9, no growing pig was pathogen-

free (n=29). There was still a high prevalence at the second sampling time in comparison to the other herds with 81.5%. Nearly the same results were achieved by herd 12 with 100% (n=31) carriers of *Campylobacter* spp. at the beginning of fattening period and 80.6% at the end of growing time. In every herd the prevalence decreased from the first sampling time to the second. Only in herd 3 did the prevalence increase from 75.9% to 86.2%.



¹ C. total = *C. coli* and/or *C. jejuni*

² herd = 29 to 31 sampled pigs per herd

Fig. 3: Prevalence of *Campylobacter* spp. in the fattening pigs at herd level (Prävalenz von *Campylobacter* spp. bei Mastschweinen auf Betriebsebene)

Risk factors

For the statistical risk factor analysis in the fattening unit, 716 results from the bacteriological examination were evaluated in context with the questionnaire data from the twelve fattening herds. Twenty factors were tested regarding their influence on the prevalence of *Campylobacter*. Significant effects were shown for the following factors: sampling time, number of fattening places, mixed farming, floor space design, feed origin, antibacterial and anthelmintic treatments (Table 2).

Over the fattening period the *Campylobacter* spp. prevalence decreased. At the beginning the odds ratio increased by a factor of 4.46 (Table 2).

The risk factor fattening places per herd was differentiated between farms size under 1000 pigs and alternatively over 1000 pigs. The bacteriological results show that pigs from farms with less than 1000 fattening places had a prevalence of 80.0% and those from larger farms a prevalence of 74.3%. The chance to isolate *Campylobacter* spp. from pigs from smaller herds increased by a factor of 1.44.

Housing in separated stalls is another preventive influence. When the animals on mixed farms were kept in separated stalls the chance of a positive bacteriological result decreased (OR=0.61).

Pigs which were kept on a plan floor without bedding had the highest prevalence in comparison to the other flooring systems. In this housing system, the chance of obtaining a positive result was highest.

Table 2

Significant risk factor and further risks factors: fattening unit (Signifikante Risikofaktoren und weitere Einflussfaktoren bei Mastschweinen)

risk factor	p-value	prevalence (%)	OR ¹	95% CI ²
date				
<u>sampling time</u>	<.0001			
growing pigs		89.2	4.64	3.11-6.93
finishing pigs		64.7	1	-
herd organisation				
<u>number of fattening places</u>	0.052			
< 1000 places		80.0	1.44	1.00-2.08
> 1000 places		74.3	1	-
<u>mixed farming</u>	0.015			
stall separated		74.6	0.61	0.41-0.92
stall not separated		82.0	1	-
housing system and forage				
<u>floor space design</u>	0.001			
fully slatted floor		74.4	0.35	0.20-0.95
<50% slatted floor		74.8	0.56	0.32-0.97
plan floor without bedding		84.7	1	-
<u>feed origin</u>	0.001			
own forage		70.3	0.41	0.24-0.68
purchase forage		79.4	1	-
health				
<u>antibacterial treatment</u>	0.028			
yes		74.6	0.66	0.45-0.96
no		79.7	1	-
<u>anthelmintic treatment</u>	0.003			
yes		83.9	1.99	1.25-3.18
no		74.8	1	-
source³				
own piglets		73.3	0.26	0.09-0.75
steadier farrowing herds		76.1	0.32	0.13-0.76
purchase breeding herds		90.3	1	-
feed consistency³				
meal		70.3	0.63	0.42-0.96
granule		81.0	1.23	0.60-2.54
pellets		78.0	1	-
blank dwell time³				
>10 days		90.5	3.53	1.82-6.86
<10 days		74.5	1	-

¹ odds ratio² 95%-confidence interval³ further risk factor in the fattening unit

An antibacterial treatment at the beginning of the fattening period was implemented on seven herds. The following antibiotics were used for this treatment: Amoxicillin, Tetracycline and Sulfonamide. The chance of a positive finding decreased when the animals were treated with antibacterial substances during this time period (OR=0.66). On four herds, anthelmintics were used at the beginning of fattening period. The appliance of Ivermectin, Flubendazol and Levamisolhydrochlorid was adopted for

deworming. The chance of obtaining a positive result rose by a factor of 1.99 when anthelmintics were administered.

Further risk factors “source of piglets”, “feed consistency” and “blank dwell time” had an influence on the prevalence of *Campylobacter* spp., too. The chance of obtaining a positive result from the bacteriological investigation was smaller from fattening pigs in a closed herd system (OR=0.26). Furthermore, the following cases were preventive: feeding meal (OR=0.63) instead of granule or pellets and blank dwell time under 10 days.

Discussion

The results from the present study prove that *Campylobacter* spp. are of increasing importance in farrowing and fattening units: high prevalence of *Campylobacter* spp. were found in suckling, growing and finishing pigs (WEHEBRINK, 2006). Other studies also confirm these results (KASIMIR, 2005; GAULL, 2002).

The occurrence of *Campylobacter* spp. in subsequent samples of pigs and sows was often variable in this analysis. As known from further studies the *Campylobacter* spp. prevalence may vary because the physiological status of the animal and external factors can influence the intestinal flora. The ability of *Campylobacter* spp. to colonise the intestinal tract of pigs is probably subject to the various factors influencing the colonisation resistance of the gut (RUCKEBUSCH et al., 1991). Furthermore, the virulence of the *Campylobacter* spp. strains (re)infecting the pigs may also alter the bacteriological results (WEIJTENS et al., 1999).

The prevalence estimates on basis of bacterial findings must be questioned critically. Because of the intermittent shedding at animal level the bacterial detection in faecal samples can create a false image of the prevalence at herd level. Additionally, during sampling and laboratory processing, the pathogen's sensibility to environmental influences can decrease the detection rate.

The bacteriological analysis showed that in some herds as far as 100% of the pigs had contact with *Campylobacter* spp.. In contrast to YOUNG et al. (2000), a successful abatement strategy can be doubted due to high general prevalence and the infection of piglets during the first weeks of life.

Based on the zoonotic directive (Nr. 2160/2003), a monitoring for *Campylobacter* spp. is mandatory. It should take place at an adequate stage of the food chain. Control has to be directed primarily at the prevention of colonisation of farm animals by means of the implementation of Good Hygienic Practice (GHP), biosecurity measures and husbandry practices incorporating Hazard Analysis Critical Control Point (HACCP) based on risk management systems (WHYTE et al., 2002). Because of this, the objective of this study was to obtain more information about the risk factors influencing the prevalence of this pathogen. As a result of the small sample size in the farrowing unit, it was not possible to perform a risk analysis which yielded significant conclusions. In the fattening unit the attention was focused additionally on risk factors which do not reach the significant limitation of the 5% probability error because of the small sample size. Effects which exceeded the housing and management factors were not acquired in the questionnaire and could not consequently be regarded in the evaluation. Because of this the results should only be regarded as tendencies.

One important influencing factor could be the sampling time. Because of the steady state of immunity the chance of a positive *Campylobacter* spp. result is higher in

growing pigs than to finishing pigs. Additionally, transport stress, changing the forage and status conflicts can raise the faecal shedding of this pathogen in growing pigs.

In contrast to recent studies, risk factor analysis in the fattening unit demonstrated a significant influence on the *Campylobacter* spp. detection rate for the “number of fattening places”. The chance of obtaining a positive *Campylobacter* spp. result is higher when animals are held in smaller herds (<1000 places). This result did not conform to GAULL (2002). He detected that the factor “number of animals” hardly has any influence on *Campylobacter* spp.-positive animals.

Separating the herds in “mixed farming” is a useful method to decrease pathogen transmission. In contrast to our study, BOES et al. (2005) could not assert this effect: investigation of the occurrence and diversity of *C. jejuni* infections in finisher pigs in herds with combined cattle or poultry production and herds only producing pigs showed no evidence of transmission of *C. jejuni* from cattle or poultry to pigs in mixed production herds. Herd prevalence of *C. jejuni* was 8.3%, whereas *C. jejuni* and *C. coli* were isolated from 0.8% and 92.0% of pigs, respectively. In mixed production herds, *C. jejuni* predominated in cattle (42.7%) and poultry (31.6%), whereas *C. jejuni* was only isolated from 1.3% to 2.5% of pigs in these herds.

A lower *Campylobacter* spp. detection rate is not promoted by a plan floor without bedding and purchase forage. One reason for the higher prevalence in housing systems with plan floor is the intensive contact of the pigs with their faeces for a longer time. With regard to purchased forage, the origin is often uncertain: whether the forage comes directly from the forage producer or whether several forage chandlers are interposed, increasing the risk of contamination, remains often unknown.

A further result from the questionnaire analysis was that an arranged antibacterial treatment but no anthelmintic treatment was preventive against *Campylobacter* spp. infections. This results must be questioned critically because it is not known first which health status in detail can be found in the different herds and, second, what the antimicrobial resistance of *Campylobacter* spp. is. Further studies will be needed to explain these two risk factors.

Despite the fact that forage in granule form is heated during the manufacturing process, the chance of obtaining a positive *Campylobacter* spp. result rose by a factor of 1.23 in this form of forage feeding.

The fact that a blank dwell time under ten days is better for the pathogen prevalence than a blank dwell time over ten days can be related to recontamination after disinfection and cleaning.

Other studies found risks factors which could not be proven in this study. For example, GAULL (2002) discovered that a factor such as different “husbandry” hardly has any influence on *Campylobacter* spp.-positive animals. “Feed” and “number of pig delivering farms” are not risk factors either (WEIJTENS et al., 1993). SCHUPPERS et al. (2005) detected that important risk factors contributing to the prevalence of resistance strains were shortened tails, lameness, skin lesions, feed without whey, and *ad libitum* feeding. Multiple antimicrobial resistance was more likely in farms which only partially used an all-in-all-out system, or a continuous-flow system compared to a strict all-in-all-out animal-flow. Presence of lameness, ill-thrift, and scratches at the shoulder in the herd also increased the odds for multiple resistance. Thus, the results from SCHUPPERS et al. (2005) showed that on finishing farms which maintained a

good herd health status and optimal farm management the prevalence of antimicrobial resistance was also more favourable.

In the present study, only a few factors could be identified as potential risk factors. For further clarification of risk factors comprehensive assessment and transmission devolution studies are required.

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