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Original Contribution

EPIDEMIOLOGICAL CHARACTERISTICS OF POST-WEANING DIARRHOEA ASSOCIATED WITH TOXIN-PRODUCING *ESCHERICHIA COLI* IN LARGE INTENSIVE PIG FARMS

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ABSTRACT

Post-weaning diarrhoeas are a common health problem in intensively reared pigs. The present report presents data about their prevalence in 8 intensive farms from various regions in Bulgaria during 2005–2009. A total of 898 faecal or intestinal content samples were collected from pigs in the period after weaning to their arrival in fattening herds and submitted to bacteriological examination. A single microbial agent was detected in 57.5% of samples, and two or more agents in the other. They belonged to 10 taxonomic categories.

Toxin-producing *E.coli* belonging to the enterotoxigenic (ETEC) and enterohaemorrhagic pathovar (EHEC; VTEC) were detected in 66.1% of 619 isolated and identified strains. Out of them, 87.4% possessed the adhesion factor F18 while the rest – adhesin of the F4 type.

E.coli-associated diarrhoeas appeared mainly in the first 3–4 weeks after weaning, most commonly during the second weeks. That is when the highest incidence has been detected. They were caused by both toxicogenic *E. coli* as well as by a number of predisposing factors. In surveyed farms, they were most commonly related to feeding regimen and feeding rations. The prevalence varied from 14.1 to 29.6%. The total morbidity rate was between 19.2 and 51.5%, and the lethality – from 1.22 to 11.73%.

Post-weaning diarrhoeas associated with enterohaemorrhagic *E. coli* were prevalent over the whole year but higher morbidity rates were observed during the cold and wet months of the year.

Key words: weaned pigs, post-weaning diarrhoe, ETEC, VTEC, prevalence, incidence, lethality, seasonal prevalence

INTRODUCTION

Gastrointestinal disorders in pigs are a great challenge to intensive pig farming. They cause substantial economic losses related to mortality, stunted growth and prolonged time for reaching slaughter weight (1, 2). They are especially frequently seen around weaning. Weaning causes stress to pigs due to the simultaneous effect of several physiological, psychological and ecological factors – introduction of adult feed, separations from sows, new environment, forced living together with other pigs in large groups etc. (5). Yet, the microbial factor plays an essential role in of post-weaning diarrhoeas (3, 4).

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The etiology of diarrhoeas in the early post-weaning period is related to the so-called enterotoxigenic (ETEC) and enterohaemorrhagic or verocytotoxin and shiga toxin producing *E.coli* - EHEC; VTEC, SLTPEC. (5,6). The first possess usually adhesion antigens of the F4 type (K88ab, K88ac, K88ad), while the other – of the F18 type that causes the so called oedema disease (7, 8, 9).

Numerous risk factors were identified for the onset of these two forms of coliinfection. They are usually related to feed and the type of diet, but overpopulation, high environmental humidity and the weaning stress are also very important. (6, 10, 11, 12)

The control of diarrhoeas requires a permanent epidemiological control with hazard analysis and evaluation. This could however occur only

if the most important epidemiological traits of post-weaning diarrhoeas are acknowledged through both retrospective analyses and surveys upon epidemic outbreaks. (2)

The purpose of the present study was to follow out the epidemiological traits of diarrhoeas in pigs during the first post-weaning weeks with emphasis on the leading risk factors and quantitative analysis of epidemiological parameters.

MATERIAL AND METHODS

Eight intensive pig farms from 5 regions in Bulgaria were included in the survey. Investigations were carried out in the period 2005–2009 on 98 lots with a total number of 29,450 pigs at the age of 28 to 75 days.

Bacteriological investigations included inoculations of faeces of live pigs (anal swabs) and inoculations of intestinal mucosa with specific alterations, intestinal content and mesenteric lymph nodes from carcasses.

A total of 898 samples from recently weaned and fattening pigs were collected, both anal swabs from live pigs and carcass intestinal content.

Microbial agents were isolated and indentified on solid agar and liquid nutrient media as followed: blood agar (Bulbio base, NCIPD), Mc Conkey Agar (Bulbio, NCIPD) –for enterobacteriae, Campylobacter selective broth and agar, Yersinia selective agar (Oxoid).

Isolates were identified on the semiautomated system CRYSTAL with strips Crystal Enteric Nonfermenter, Crystal Gram-positive Kit and Streptocard Acid Kit (Becton Dickinson).

The serotype of *E. coli* isolates was determined by agglutinating sera by slide agglutination. The type of fimbrial adhesion antigens was tested in agglutination test with specific sera.

Brachyspirae were detected by native microscopy of fuchsin-stained imprint preparations made of intestinal tract lesions, and by culturing of faecal swabs or carcass intestinal swabs. The samples were placed in the Amies transport medium. Inoculations were done on trypticase soy agar with 10% sheep blood and antibiotic supplement of 200 mg/l spectinomycin, 50 mg/l vancomycin, 12.5 mg/l rifampicin and 12.5 mg/l colistin (Sigma Aldrich). Incubation was performed aerobically at 37 ^{o}C over 3–6 days. The identification was carried out as per Felström and Gunnarson, 1995 (13).

Retrospective epidemiological surveys and in-situ surveys evaluated the presence and effects of most important risk factors in each farm. Morbidity rate, mortality and lethality rates as well as the seasonal pattern of post-weaning diarrhoeas caused by EHEC and *Brachyspira* spp. were determined. The morbidity rate was expressed as ratio of the number of diseased pigs to the total number of pigs in the lot by the time of weaning. The mortality rate was calculated as percentage ratio of pigs dead from diarrhoea (only with confirmed infection) to the total number of pigs in the post-weaning lot and lethality – as ratio of the total number of pigs dead to the number of diseased pigs (pigs with diarrhoea).

RESULTS

1. Bacteriological studies

Bacteriological tests of 898 samples revealed microbial agents in 758 or 84.5%. Only one microbial agent was present in 436 (57.5%) samples. *Brachyspira* spp. was detected in 112 samples (14.8 %), and alterations specific for ileitis (*Lawsonia intracellularis*) were present in 17 samples (2.2%). The other 140 samples (15.6%) were negative, i. e. no bacterial finding was detected.

Six hundred nineteen toxin-producing *E. coli* strains were detected in 66.1% or 501 samples. Out of them, 438 (87.4%) possessed the adhesion factor F18, and 63 (12.6%) – F4 adhesin. The taxonomic affiliation of the other 118 isolates is presented in **Table 1.**

According to data about the etiology of bacterial infections in recently weaned and fattening pigs with diarrhoeic syndrome, E. coli strains producing a shiga-like toxin and possessing the F18 adhesion antigen were prevailing. On the second place came the representatives of the genus *Brachyspira,* whereas the prevalence of species-adapted *S.* Choleraesuis isolates was only 6.6%. This determines the post weaning diarrhoea, associated with enterohaemorrhagic *E. coli* as a leading intestinal disease during the period from weaning to fattening herds arrival. Questionnaire surveys showed that they appeared most frequently during the first 3 post-weaning weeks with peak during the second week. After this period, their incidence sharply decreased. In 92% of *E. coli* strains isolated from diarrhoeas occurring during the

first post-weaning week, the fimbrial antigen F4 was detected while adhesin F18 was categorically predominating among isolates from later post-weaning periods. It was detected as the only antigen among strains isolated in diarrhoea cases after the 3rd post-weaning week.

Table1. *Microbial species detected in intestinal content and faeces of recently weaned and fattening pigs aged 28–75 days with diarrhoeic syndrome.*

	Microbial species	Number of isolates	%
1	*Toxicogenic Escherichia coli*	501	66.1
2	*Brachyspira* spp.	112	14.8
3	*Salmonella* choleraesuis	50	6.6
4	*Clostridium* spp	31	4.1
5	*Lawsonia intracellular is*	17	2.2
6	*E. coli (SEPEC)*	14	1.8
7	*Salmonella* Enteritidis	11	1.5
8	*Campylobacter coli*	6	0.8
9	*Campylobacter jejunii*	3	0.4
10	*Salmonella* spp.	4	0.5

2. Epidemiological investigations

The data about morbidity, mortality and lethality rates of EHEC-associated diarrhoeas in the 8 pig farms are shown on **Table 2**. The average morbidity rate was 23.59%, ranging from 17.5% to 51.53%. The lethality in the same farms was between 9.5% and 33.6%, 21.7% on the average.

Table 2. *Prevalence, mortality and lethality rates in recently weaned pigs with postweaning diarrhoea caused by toxin-producing E. coli in 8 intensive pig farms for 2005–2008.*

Pig farm	Lots surveyed	Number of pigs per lot	Prevalence, %	Lethality %	Mortality %
1	8	230±10.3	46.5	33.6	11. 73
2	7	420±12.1	19.2	27.4	1.42
3	12	180±11.4	21.6	22.8	2.77
4	9	400±5.4	17.5	9.5	2.18
5	12	250±10.1	18.1	18.5	1.77
6	21	180±12.3	44.3	27.1	3.07
7	8	280± 6.3	19.7	14.7	1.22
8	11	240±5.3	51.5	19.5	2.98
Average	**Σ=88**		**23.5**	**21.7**	**3.39**

It should be specified that these were the average parameters on a farm level, but there were considerable differences among the lots within a farm. That is why we have followed out the incidence of EHEC-associated diarrhoeas in 3 affected farms at a time as well the lethality and cumulative mortality at the lot level. The weekly incidence over 8 weeks was determined from the weaning day to the arrival of piglets in fattening sectors. Data from this comparative investigation are shown on **Fig. 1**.

In the three farms, coliinfections with diarrhoeic syndrome appeared mainly within the first 3–4 post-weaning weeks and rarely after that time. The incidence was especially high during the second week after the transition of piglets from milk to adult compound food. The incidence by that time ranged from 1.7% to 16.3%. This means that over a relatively short period of time (one week), up to 1/5 of the susceptible pig population could be involved in the epidemics.

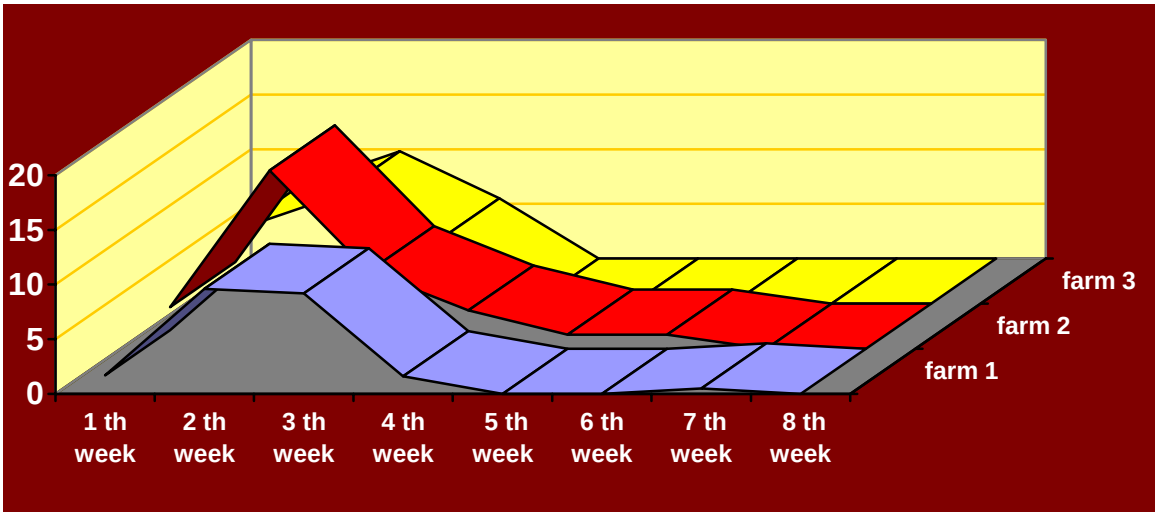

Fig. 1. Dynamics of the weekly incidence of EHEC-associated post-weaning diarrhoeas in three different affected pig farms

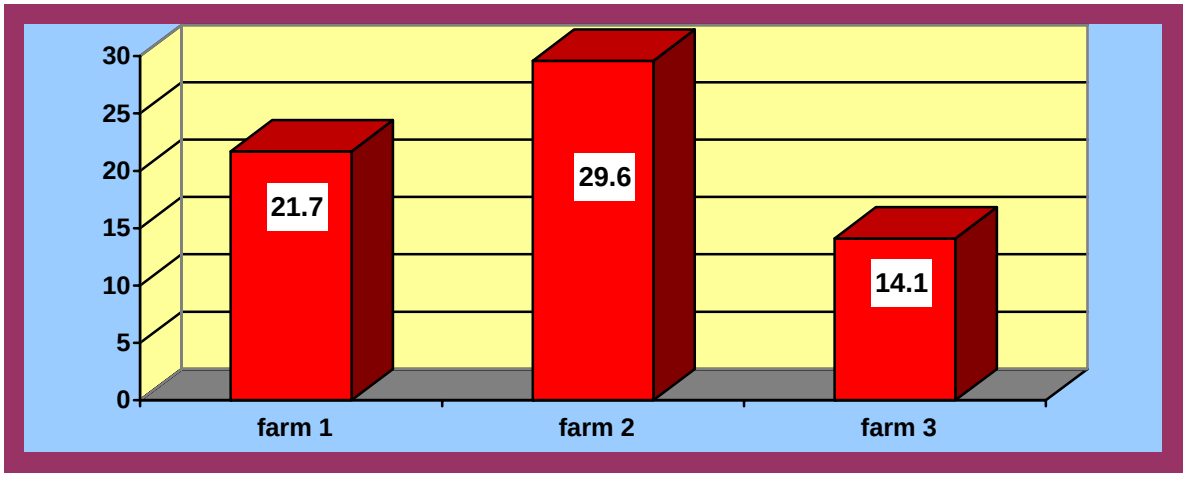

Fig. 2. Prevalence of EHEC-associated post-weaning diarrhoeas in pigs in three intensive pig farms in 2009.

The prevalence as detected by the end of the period prior to moving ranged between 14.1% and 29.6% (**Fig.2**).

The availability of microbial factor with pathogenic potential is not the only condition for diarrhoeas to appear. Predisposing risk factors are not less important. Their relative contribution in the different farms, expressed by a cross-score system is presented in the next table.The table shows that in some farms (№№ 1, 6, 8) feeding and diet type had a leading role. In them, the maximum prevalence has been observed. In some instances, the feed quality was important (farm № 6), whereas in others – unbalanced diet was more important (farms №№ 1, 3). Most frequently, diarrhoeic outbreaks were however observed in recently weaned pigs that were fed two times daily with a specified amount of feed instead of being fed *at libitum* – farms №№ 1, 3, 6, 8.

RISK FACTOR	FARMS							
	1	2	3	4	5	6	7	8
No metaphylaxis prior to weaning	+	-	(+-)	-	-	+	-	+
Sudden weaning	-	-	-	+	-	+	-	-
Unbalanced diet	+	(+/-)	+	(+/-)	-	-	-	-
Undifferentiated feeding	+	-	-	-	-	+	-	+
Feeding *ad libitum*	-	+	-	(+/-)	+	-	+	-
Feeding two times daily with limited amount of feed	+	-	+	-	-	+	-	+
Overpopulation	-		+	+	(+/-)	-	+	(+/-)
Poor hygiene	-	+	-	-	-	-	+	-
Excessive humidity	-	+	-	-	(+/-)	-	+	-
Impaired temperature regimen	-	+	-	-	+	+	-	+
Current disinfection	-	-	(+/-)	-	-	(+/-)	+/-)	-

Legend:
(-) the risk factor is absent
(+/-) the risk factor is occasionally present
(+) the risk factor is always present

Overpopulation (farms №№ 3, 4 and 7) was also important, as well as excessive humidity (farms №№ 2 and 7). The lack of preventive supplementation of prestarter rations with antimicrobial drugs could play a role as well (farms №№ 3 and 8).

Neglecting current disinfections could also has an effect as it favourizes the persistence of high microbial contamination rates. For pigs housed on slats, the influence of this factor is relatively lower.

The stable presence of these predisposing causes as well as the circulation of highly toxicogenic *E. coli* strains in premises for growing pigs makes post-weaning diarrhoea a persistent problem for most of surveyed farms. Their prevalence was observed on an annual basis although being higher during the cold and wet months. This was depicted on **Fig. 3** that shows the monthly dynamics of post-weaning diarrhoea prevalence for all farms.

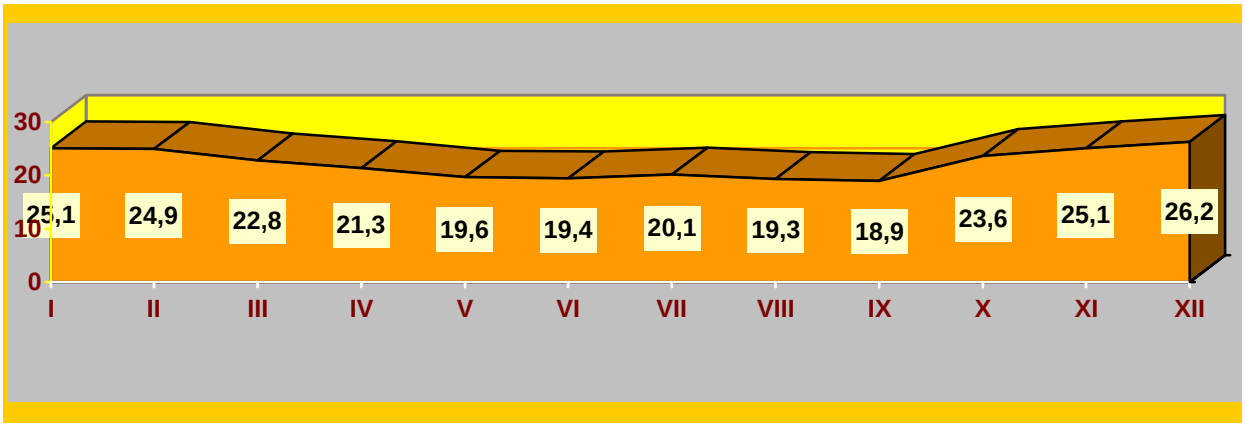

Fig. 3. Monthly incidence of EHEC-associated infections manifested as post-weaning diarrhoea in 8 intensive pig farms

DISCUSSION

Our investigations allowed to assume that during the first 2–3 post weaning weeks, toxin-producing E. coli that possess the adhesion factor F4 were most involved in the etiology of diarrhoeas. They were however effective only provided that several predisposing causes are present. In some farms, a single risk factor is identified but in most cases they are several and act simultaneously and in synergy. Factors related to the change in feed and feeding regimen predominated. Such conclusions were also made as far back as in 1979 by Leece et al., who found out a considerable effect of food type – liquid or dry – on diarrhoeas' incidence. The authors recommended intake of liquid food in a controlled amount and regular feeding as conditions for reduction of both frequency and

severity of diarrhoeas in recently weaned pigs and particularly during the first post-weaning week (14).

The food intake after weaning is also an important precondition. According to Rantzer et al. (1996) the reduced amount of daily food intake during the first week decreased the incidence of intestinal disorders (11). The opposite opinion was expressed by Madec et al. (1998) supporting that the limitation of feed carried a greater risk due to the enhanced motility of the small intestine. In this study, we also found out that the prevalence of post-weaning diarrhoeas was lower in farms with *ad libitum* feeding practices (5).

Similarly to Laine et al. (2008), we found out that the reduction of feeding frequency to twice a day was more dangerous with regard to the appearance of intestinal disorders compared to farms when feeding was offered *ad libitum*. (10)

Overpopulation of pigs was also often encountered. In some of farms excessive humidity and poor hygiene played a significant predisposing role.

The permanent presence of one or more of these factors and especially their synergistic action results in diarrhoea outbreaks (15). As could be seen from this survey, the simultaneous presence of several risk factors could result in disease in more than half of susceptible population. Moreover, such outbreaks were observed all year round.

All these facts require a complex approach to control post-weaning diarrhoeas, that should include a precise feeding schedule and ration, maintenance of appropriate hygiene level and microclimatic conditions in premises and last but not least, metaphylaxis with proper antimicrobial drugs.

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