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ETIOLOGY, EPIDEMIOLOGICAL MONITORING AND THERAPEUTIC CONTROL OF BACTERIAL ENTERITIS AMONG SUCKLING PIGS IN BULGARIA

M. LYUTSKANOV & V. URUMOVA

Department of Veterinary Microbiology, Infectious and Parasitic Diseases, Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria

Summary

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An analysis of the incidence of neonatal bacterial enteritis was performed in 12 intensive pig farms from different regions of Bulgaria. A total number of 380 faecal samples from live piglets and intestinal content of fresh carcasses were examined. Bacterial findings were observed in 89.2% of samples, and isolates belonged to 11 taxonomic categories. Enterotoxigenic *E. coli* (ETEC) predominated (58.1% of all samples), followed by *C. perfringens* with 14.1%. Colienterotoxicosis was observed mainly during the first week of piglets' life and most commonly affected 50–75% of pigs from diseased litters. The mean lethality was 48.3%, and cumulative mortality – 18% for the respective epidemic outbreak. Autumn-winter and winter-spring prevalence was higher. The sensitivity of ETEC isolates towards gatifloxacin, levofloxacin and enrofloxacin, colistin, neomycin and gentamicin was preserved. A relatively high resistance was established towards doxycycline (55.4%) and spectinomycin (42.4%).

Key words: enteritis, enterotoxigenic *E. coli*, lethality, morbidity, seasonal pattern, resistance

INTRODUCTION

Gastrointestinal diseases are among the major causes of death for suckling piglets (Morin *et al.*, 1983; Holland, 1990; Sanz *et al.*, 2001). In most cases, they are etiologically related to some kind of microbial agent – bacteria, viruses, protozoa. Many of intestinal tract infections are mixed or associated (Johnson *et al.*, 1992; Wieler *et al.*, 2001; Yaeger & Hoff, 2002).

The infection of newborn pigs with pathogenic bacteria occurs during the birth itself or during the first hours/days of life. The commonest route of infection is the faecal-oral one. The presence of risk factors is also important, among which most often is the insufficiency of milk or excessive nursing (Bergeland & Henry, 1982; Sanz *et al.,* 2001). Hygiene of the mother's mammary gland is also important (Holland, 1990). Despite there is plenty of data about the structure of the microbial flora, involved in the diarrhoea syndrome, its investigation is still important in order to rationalize treatment and prevention.

Bacterial enterites occur with variable intensity depending on the etiological agent and the presence of predisposing causes. They affect a variable number of piglets from the litter and spread among litters and rooms at different rates (Armstrong & Cline, 1977; Bergeland & Henry, 1982). Knowledge of the causes, peculiarities and the epidemic process in bacterial enterites is the basis of proper control (Wieler *et al.*, 2001). Establishing the specifics and principles in intestinal infection epidemiology would help make these measures more efficient. In this relation, great significance is attributed to healing or metaphylactic treatment with antimicrobial substances, accompanied by monitoring for build up of resistances against them.

The goal of the current study is to analyze the species content of the microbial factor responsible for neonatal diarrhoeas in pigs, establish its reactions towards antimicrobial substances, and clarify some basic epidemiological features of massive intestinal infections with regard to optimizing both prevention and control.

MATERIALS AND METHODS

The study included 12 pig farms of the intensive type – 7 in northern and 5 in southern Bulgaria. The examinations were performed in the period between the years 2005 and 2009. In all analyzed cases there were epidemic outbreaks of gastrointestinal diseases in suckling pigs, accompanied by diarrhoea syndrome.

Bacteriological studies included cultivations of faeces from living pigs (anal swab samples), as well as cultivations from intestinal mucosa and contents obtained from pig carcasses. A total of 380 samples were examined, including 155 anal swab samples from living animals and 225 samples of intestinal content from fresh carcasses. Samples distribution per years is presented in Table 1.

In order to isolate and identify the bacterial etiological agents, the following solid agar and liquid nutrient media were used: blood agar (basis - Bulbio-National Centre of Infectious and Parasitic Diseases; NCIPD, Bulgaria), McConkey agar (Bulbio-NCIPD) – for enterobacteria, Campylobacter selective medium and agar, Yersinia selective agar (Oxoid, UK).

Identification of the isolates was done with the semi-automatic CRYSTAL system, using Crystal Enteric Nonfermenter strips, Crystal Gram-positive Kit and Streptocard Acid Kit (Becton Dickinson, USA).

The isolates' serotypes were determined with saturated agglutinating anti-*Salmonella* and anti-*E.coli* sera in slide agglutination. Slide agglutination with specific antisera was used to detect the fimbrial antigens F4 and F5.

In order to determine the susceptibility

Table 1. Distribution of samples by years

Samples	Years					Total
	2005	2006	2007	2008	2009	
Anal swab samples from suckling pigs	36	47	48	35	25	191
Intestinal content from suckling pigs' carcasses	30	36	62	28	33	189
Total	66	83	110	63	58	380

of isolated and identified strains towards antimicrobial substances, the disk-diffusion Bauer-Kirby test was used in accordance with the requirements of Clinical Laboratory Standard Institute – Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals – Approved Standards – M-31-A3, Vol. No 8, Replaced No 31-A2, Vol. 22, No 6.

Antibiogrammes were performed on the Mueller-Hinton agar (NCIPD, Sofia). Disks and tablets of 10 antimicrobial substances with concentrations of chemotherapeutical substances are presented in Table 2.

Table 2. Antibacterial disks used in testing of bacterial isolates from suckling pigs

Antibacterial disk	Code	μg/disk
Gentamicin*	G	10
Neomycin**	N	30
Spectinomycin***	Spt	30
Doxycyclin***	D	30
Tiamphenicol*	C	30
Colistin***	Col	50
Flumequin***	Flu	5
Enrofloxacin****	Enr	5
Levofloxacin*	Lev	5
Gatifloxacin*	Gat	5

* produced by NCIPD; ** produced by BBL; *** produced by CEVA; **** produced by Bayer.

Epidemiological analysis included the indicators of lethality, mortality and prevalence, as well as seasonal occurrence.

RESULTS

After the bacteriological studies were done, bacteria were found in 339 out of 380 samples from suckling pigs or 89.2%. Among them, in 303 samples (89.4%) only one microbial species was found, whereas polymicrobial infection was found in 36 samples (10.6%) – 27 samples with two microbial species and 9 samples with three species. A total of 382 bacterial strains were isolated and identified, belonging to 12 different taxonomical categories.

Table 3 presents the summarized data on the established microbial species in suckling pigs for the entire experimental period and their relative share.

The data show that the etiology of sucking piglets with diarrhoea symptomatology was strongly dominated by the enterotoxigenic *E. coli* (ETEC), which were proved in 58.1% of the samples. Second after them were the representatives of *Clostridium* spp., more specifically *C. perfringens* type A and type C, which made up 14.1% of the isolates. The septicaemic *E. coli* strains made up 8.6% of the samples, followed by *Campylobacter coli* – 5.2 %. Enterococci were detected relatively often (4.2%), yet their participation in the etiopathogenesis of diarrhoeas should be interpreted carefully due to their resident nature. *Salmonella* representatives were isolated from 4.9% of the samples, dominated by the *Salmonella enterica* subsp. Enteritidis serotype.

It should be noted that only 17.5% of the enterotoxigenic *E. coli* isolates could be found in associations with other bacterial agents – clostridia, campylobacteria, enterococci, and *Y. enterocolitica*. In the other 82.5%, colibacteria of this pathovar could be isolated in monocultures. This established colienterotoxicosis as a leading intestinal illness during the neonatal period of pigs.

The epidemiological analysis indicated that in the cases of proven colienterotoxi-

Table 3. Microbial species, isolates from faeces and intestinal content of suckling pigs

Microbial species	Number of isolates	Percentage of isolates
*Escherichia coli (*ETEC*)*	222	58.1
*Escherichia coli (*SEPEC*)*	29	7.6
Clostridium perfringens A and *C* type	54	14.1
Clostridium difficile	6	1.6
Salmonella Enteritidis	6	1.6
Salmonella Typhimurium	3	0.8
Salmonella spp.	8	2.1
Campylobacter jejunii	12	3.1
Campylobacter coli	20	5.2
Yersinia enterocolitica	4	1.0
Enterococcus spp.	10	2.6
Streptococcus suis	8	2.1

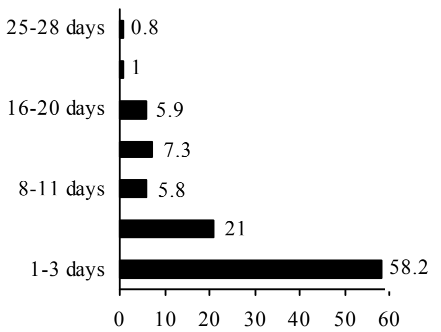

Fig. 1. Age-related incidence (%) and time course of appearance of neonatal colienterotoxicosis (ETEC) in pigs from birth to weaning.

cosis the clinical symptoms occurred primarily during the first third of the nursing period. Fig. 1 presents the prevalence of colienterotoxicosis in pigs from birth to weaning.

It is apparent that this infection occurred mostly during the first week of life (79.2%) with more than half of the cases occurring within the first 3 days (58.2%).

The epidemiological data show that in more than 80% of the cases about 1/2 to 2/3 of the pigs in the litter were affected. In about 15% of the cases the percentage of diarrhoea-affected pigs was within 75% and 100%, and only in 5% of the cases the number of affected pigs was less than half of the litter (Fig. 2).

Table 4 shows data on lethality of colienterotoxicosis-affected suckling pigs. The mean lethality in the 12 examined

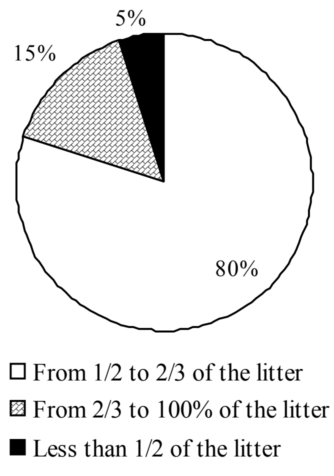

Fig. 2. Relative share of morbidity rate caused by ETEC in the different litters.

farms was 48.3%, ranging between 19.8% and 73.1%. At the same time, the cumulative mortality rate, measured as a ratio of dead animals towards the total number of non-weaned pigs in the affected sector, varied between 9.6% and 28.3%, with a mean value of 18%.

The summarized data on the monthly distribution of illness, lethality, and mortality in the 12 examined farms are presented in Table 5. It is apparent that morbidity was relatively higher during the autumn-winter and winter-spring seasons, compared to the summer months of the year. At the same time, lethality was higher during the hot months. The values for cumulative mortality remained relatively constant throughout all months.

Data on the *in vitro* distributions of isolated ETEC strains according to antimicrobial agents' sensitivity are presented in Table 6. The data indicate that the enterotoxigenic *E. coli* strains, isolated from suckling pigs, exhibited preserved susceptibility towards fluorinated quinolones, especially towards those of the newer

Table 4. Lethality and mortality rates from ETEC infections in examined pig farms

Pig farm	Number of outbreaks	Lethality, %	Mortality, %
1	5	66.2	22.8
2	4	43.3	17.8
3	4	29.8	16.3
4	6	50.8	21.6
5	2	73.1	15.8
6	3	68.4	21.2
7	4	38.8	11.5
8	3	42.1	18.8
9	6	39.5	18.2
10	4	56.8	14.0
11	4	51.0	28.4
12	5	19.8	9.6
Average		48.3	18.0

Table 5. Monthly distribution of morbidity, lethality, and mortality rates from ETEC in suckling pigs

Month	Morbidity rate, %	Lethality rate, %	Mortality rate, %
January	56.2	26.3	14.8
February	56.2	19.6	11.0
March	55.3	15.9	8.8
April	29.7	33.7	10.0
May	29.9	30.4	9.1
June	17.8	52.8	9.4
July	16.9	49.7	8.4
August	21.3	55.9	11.9
September	39.8	24.6	9.8
October	40.0	29.3	11.7
November	49.5	24.6	12.2
December	54.8	21.7	11.9

Table 6. Distribution of microbial isolates according to their antibacterial sensitivity

Antibacterials tested	Tested strains	Sensitive		Resistant + intermediate	
		Number (percentage)	Confidence limits	Number (percentage)	Confidence limits
Amoxicillin	220	132 (60.0)	25.6÷66.3	88 (40.0)	33.6÷46.5
Gentamicin	220	168 (76.4)	70.6÷81.7	52 (23.6)	18.3÷29.4
Neomycin	186	152 (81.7)	75.9÷86.9	34 (18.3)	13.1÷24.1
Tiamphenicol	186	136 (73.1)	66.5÷79.2	50 (26.9)	20.8÷33.5
Doxycycline	220	98 (44.5)	38.0÷51.1	122 (55.4)	50.1÷61.9
Spectinomycin	210	121 (57.6)	50.8÷64.1	89 (42.4)	36.8÷49.1
Colistin	220	206 (93.6)	90.0÷96.4	14 (6.4)	3.5÷10.0
Flumequin	180	117 (65.0)	58.9÷71.7	63 (35.0)	28.2÷42.1
Enrofloxacin	220	178 (80.9)	75.4÷85.8	42 (19.1)	14.2÷24.5
Levofloxacin	92	90 (97.8)	94.8÷99.78	2 (1.2)	0.01÷4.40
Gatifloxacin	92	90 (97.8)	94.8÷99.78	2 (1.2)	0.01÷4.40

generations – levofloxacin and gatifloxacin. The established sensitivity for both chemotherapeutics was 97.8%, and for enrofloxacin – 80.9%.

Sensitivity towards colistin was also high (93.6%), as well as towards the aminoglycosides neomycin (81.7%) and gentamicin (76.4%). Then came the sensitivities towards tiamphenicol (73.1%), spectinomycin (57.6%), flumequin (65.0%), and amoxicillin (60.0%). The highest resistance was established for doxycycline and spectinomycin − 55.4% and 42.4% of the isolates, respectively.

DISCUSSION

The data gathered in the study showed a vast variety of microbial species, which could cause enzootic outbreaks of illnesses among suckling pigs with diarrhoea syndrome. The enterotoxigenic strains of *E. coli* are clearly dominant (Guinée *et al.*, 1981; Benfield *et al.*, 1988; Holland, 1990; Nagy & Fekete, 1999). There was also a frequently detected symptom complex associated with *C. perfringens*, whose A and C types could cause the so-called necrotic enteritis (Bergeland, 1977; Waters *et al.*, 2003). More limited but not without significance was the role of *Campylobacter* spp., *Y. enterocolitica,* as well as some *Salmonella* serovars with ubiquitous behaviour (Johnson *et al.*, 1992).

The causes of the domination of neonatal colienterotoxicosis were mostly related to omissions in the hygiene of birth and nursing with colostrum, overnursing, cold or other predisposing stress factors, especially mistakes and omissions in the inoculation programme of pregnant sows and replacement pigs. This is mostly true for clostridial infections as well (Waters *et al.*, 2003).

During an epidemic outbreak the prevalence was high and most often affected more than two thirds of the litter. It was higher during the cold months of the year because cold, especially when accompanied by high humidity, deteriorates the epidemic situation. The higher lethality during the summer months, observed in nearly all examined farms, could be explained by faster dehydration as a result of diarrhoea-induced fluid loss and lower environmental humidity (Bergeland & Henry, 1982).

Successful control of coli infections in suckling pigs is closely related not only to vaccinating the mothers (passive collateral immunity) but also to a great extent to the programmes for medication control. Therefore the problem of antimicrobial reactions with field isolates is of great significance. The results show evidence of retained sensitivity towards most classes of antimicrobial agents: 4-quinolones, aminoglycosides and polypeptides. The data are close to what was established by Matteu & Martin (2000) in Spain, Choi *et al.* (2002) in South Korea, as well as Hariharan *et al.* (2004) in Canada. The data on the tiamphenicol are particularly important in light of the reports about the increasing resistance of haemolytic *E. coli*, isolated from pigs towards chloramphenicol (Bischoff *et al.*, 2002).

The data indicating relatively high levels of resistance towards spectinomycin and doxycycline could be explained mostly as a result of selective pressure caused by their non-indicated usage for individual and group treatment. It is known that they are used not only in treatment but also for metaphylaxis of dysentery (lincospectin) and/or enzootic *Mycoplasma* pneumonia (doxycycline, lincospectin), as well as for streptococci-induced and other bacterial diseases. This risk of resistant strain selection towards other antimicrobial agents necessitates more precise metaphylactic programmes and more frequent application of etiotropic measures after *in vitro* testing.

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Correspondence:

Assoc. Prof. M. Lyutskanov
Department of Veterinary Microbiology,
Infectious and Parasitic Diseases,
Faculty of Veterinary Medicine,
Trakia University,
Student's Campus,
6000 Stara Zagora, Bulgaria