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Hemolytic Porcine Intestinal *Escherichia coli* without Virulence-Associated Genes Typical of Intestinal Pathogenic *E. coli*▽†

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Testing 1,666 fecal or intestinal samples from healthy and diarrheic pigs, we obtained hemolytic *Escherichia coli* isolates from 593 samples. Focusing on hemolytic *E. coli* isolates without virulence-associated genes (VAGs) typical for enteropathogens, we found that such isolates carried a broad variety of VAGs typical for extraintestinal pathogenic *E. coli*.

Hemolytic *Escherichia coli* strains are common in the intestine of clinically healthy and diseased pigs (2, 9–12) and often do not possess virulence-associated genes typical of intestinal pathogenic isolates (iVAGs) like *est-1a*, *est-2*, *eltB*-Ip, and *faeG*, which are frequently observed among enterotoxigenic *E. coli*, or stx_{2e} and *fedA*, which are characteristic of edema disease-causing *E. coli* (9, 10). As such isolates were not the main focus of recent clinical diagnosis, knowledge of their virulence gene profile is poorly defined. Based on our initial observations (10), we assumed that such *E. coli* strains harbor high numbers of VAGs typical for extraintestinal pathogenic *E. coli* (ExPEC; eVAGs), which cause urinary tract infections, septicemia, and meningitis in both humans and animals (5). The present study assessed the frequency of hemolytic *E. coli* isolates lacking iVAGs in swine and provides a first insight into virulence gene features as an initial step toward understanding this *E. coli* group.

As depicted in Fig. 1, *E. coli* cells were obtained from feces or intestinal contents of clinically healthy and diarrheic pigs. Alpha-hemolysis of *E. coli* was tested as previously described (8). Hemolytic *E. coli* isolates from clinically healthy pigs were obtained from 132 pigs on 24 farms between 2002 and 2009 (for details, see Table S1 in the supplemental material). During a longitudinal study, hemolytic *E. coli* isolates from healthy pigs were studied over 19 months at another farm. Here, 127 randomly chosen samples from 73 pigs were collected. Samples of intestinal *E. coli* isolates from diseased pigs with enteritis or edema disease were obtained between 2007 and 2009 during routine laboratory diagnosis (Institut für Hygiene und Infektionskrankheiten der Tiere, Justus-Liebig-Universität, Giessen, Germany). The pigs (n = 1,407) were from 530 farms with unknown production status.

Hemolytic isolates were tested for the presence of iVAGs, including *faeG*, *fanA*, *fasA*, *fedA*, and *fimF41a* (coding for subunits of F4, F5, F6, F18, and F41 fimbriae, respectively) and stx_{2e}, *est-1a*, *est-2*, *eltB*-Ip (coding for toxins) by PCR as previously described (1, 6). All isolates carrying at least one of these iVAGs were excluded from further investigations. In cases in which more than one hemolytic *E. coli* isolate was isolated from a single farm, duplicate isolates, as determined by macrorestriction analysis (pulsed-field gel electrophoresis [PFGE]) (8), were excluded from further analyses (Fig. 1). Nonduplicate hemolytic *E. coli* isolates without iVAGs were then tested for eVAGs (Table 1) and classified into phylogenetic groups according to the EcoR system by PCRs previously described (3, 5, 10). Statistical analyses for comparison of the occurrence of genes between isolates from animal groups were performed using two-tailed Fisher's exact test. Analysis of the occurrence of eVAGs according to an EcoR group used one-way analysis of variance (ANOVA) with a Bonferroni *post hoc* test.

Hemolytic *E. coli* isolates were isolated from 62 clinically healthy pigs (47.0% of all samples). These 62 isolates (1 isolate per pig) belonged to 37 PFGE types, of which 32 did not carry iVAGs. In the longitudinal study, hemolytic *E. coli* isolates were isolated from 47 randomly chosen intestinal samples (37% of all samples). These 47 hemolytic isolates (1 isolate per sample) were divided into eight PFGE types, 5 of which did not carry iVAGs. One PFGE type with a unique and stable PFGE pattern and without iVAGs was detected over the 19-month sampling period in 20 different animals. Hemolytic *E. coli* isolates were isolated from 484 samples from diseased pigs (34.4% of all samples). A total of 121 isolates did not carry iVAGs. A total of 62 randomly chosen isolates—each from a different farm—were included in further analysis (Fig. 1).

The 37 isolates with a unique PFGE type from clinically healthy pigs, including the five isolates from the longitudinal study and 62 isolates from diseased pigs, were tested for

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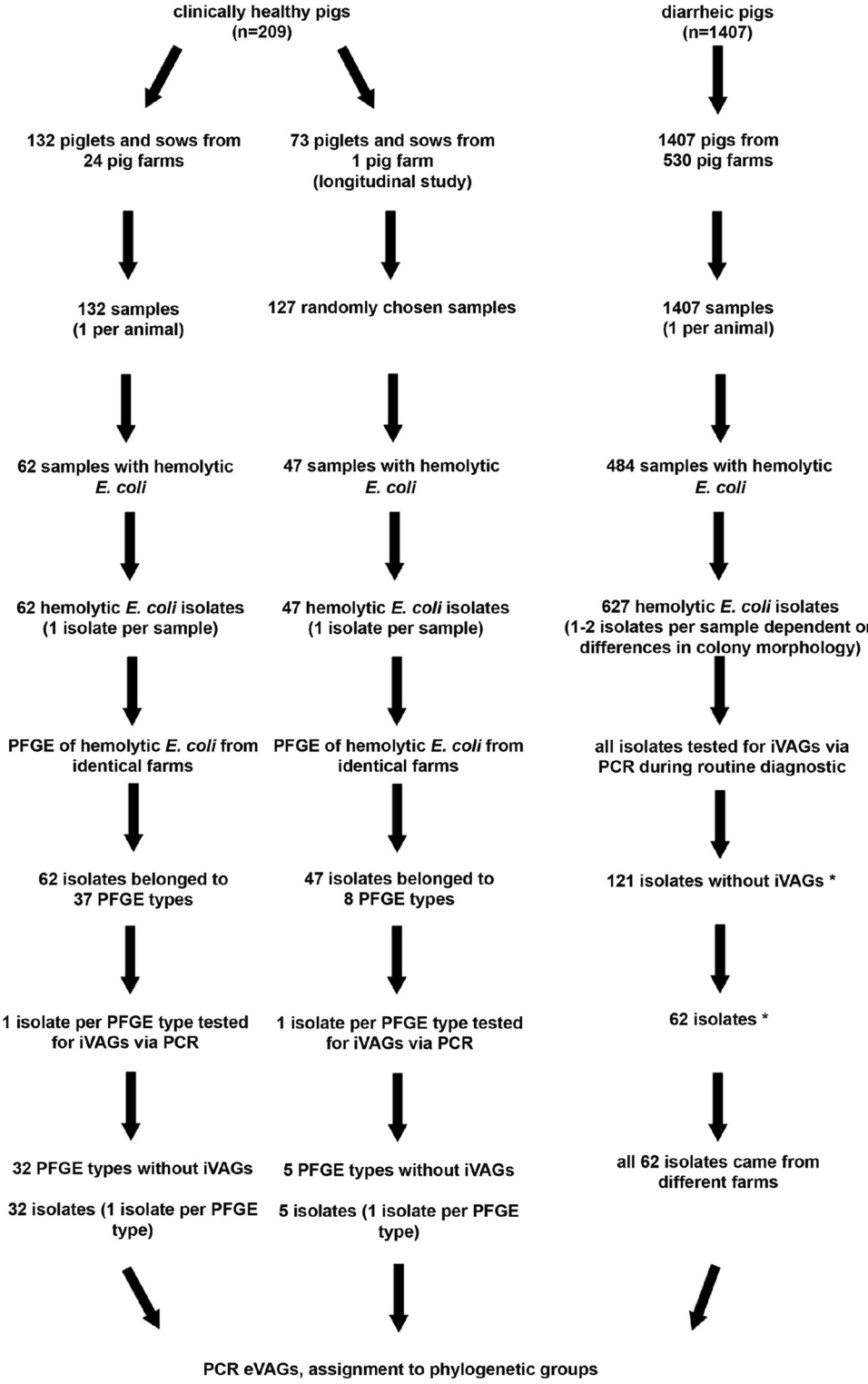

FIG. 1. Flowchart of sampling and characterization of *E. coli* isolates in this study. Clinically healthy pigs were from production units in Berlin, Brandenburg, Lower Saxony, Saxony, Thuringia, and Schleswig-Holstein, Germany. Diseased pigs were from production units in North Rhine-Westphalia, Hesse, Schleswig-Holstein, Bavaria, Lower Saxony, and Baden-Wuerttemberg, Germany, and Styria and Lower Austria, Austria. Asterisks indicate that the remaining 59 (out of 121) hemolytic *E. coli* isolates were not available as cultures and therefore could not be included in further analysis.

TABLE 1. Occurrence of eVAGs in porcine intestinal hemolytic *E. coli* and comparison to data from an already published study on porcine intestinal nonhemolytic *E. coli*[a,b]

Gene(s) or operon	Description	% of positive isolates[c]			
		Hemolytic *E. coli*[d]			Nonhemolytic *E. coli* from healthy pigs ($n = 65$)[b]
		Diseased pigs ($n = 62$)	Healthy pigs ($n = 37$)	Total ($n = 99$)	
Adhesins					
afa/draB	Afimbrial/Dr antigen-specific adhesin	0	5.4	2.0	0
fimC	Type 1 fimbriae (D-mannose-specific adhesin)	98.4	97.3	98.0	80.0
hra	Heat-resistant agglutinin	25.8*	54.1*	36.4	10.8
iha	Iron-regulated-gene homologue adhesin	0	0	0	0
papC	Pilus associated with pyelonephritis	21.0	37.8	27.3	0
sfa/focCD	S fimbriae (sialic acid-specific) and F1C fimbriae	17.7	32.4	23.2	0
tsh[b]	Temp-sensitive hemagglutinin	53.2*	18.9*	40.4	6.2
csgA	Curli fiber-encoding gene	90.3	91.9	90.0	NT
mat	Meningitis-associated and temp-regulated fimbriae	88.5	89.5	88.9	76.9
Iron acquisition					
chuA	Heme receptor gene (*E. coli* heme utilization)	16.1*	40.5*	25.3	10.8
fyuA	Ferric *Yersinia* uptake (yersiniabactin receptor)	17.7*	48.6*	30.0	16.9
ireA	Iron-responsive element (putative catecholate siderophore receptor)	6.5	8.1	7.1	0
iroN[b]	Catecholate siderophore (salmochelin) receptor	19.4*	51.4*	31.3	10.8
irp2	Iron-repressible protein (yersiniabactin synthesis)	14.5*	40.5*	24.2	16.9
iucD	Aerobactin synthesis	9.7	24.3	15.2	10.8
sitD (chromosomal)	*Salmonella* iron transport system gene	9.7	21.6	14.1	1.5
sitD (episomal)	*Salmonella* iron transport system gene	9.7	24.3	15.2	9.2
iutA	Aerobactin receptor	25.8	27.0	26.3	NT
Protectins/serum resistance					
iss	Increased serum survival	22.6	16.2	20.2	10.8
neuC	K1 capsular polysaccharide	3.2	0	2.0	0
kpsMTII	Group II capsule antigens	32.3	35.1	33.3	9.2
ompA	Outer membrane protein	100	100	100	98.5
traT	Transfer protein	85.5*	64.9*	77.8	60.0
Toxins					
astA	EAST-1 (heat-stable cytotoxin associated with enteroaggregative *E. coli*)	1.6*	18.9*	8.1	12.3
sat	Secreted autotransporter toxin	0	0	0	0
hlyA	Hemolysin A	98.4	100	99.0	0
cnf1/2	Cytotoxic necrotizing factor	16.1	24.3	19.2	NT
Invasins					
gimB	Genetic island associated with newborn meningitis	3.2	2.7	3.0	0
ibeA	Invasion of brain endothelium	3.2*	18.9*	9.1	4.6
tia	Toxigenic invasion locus in ETEC[e] isolates	0	2.7	1.0	0
Miscellaneous					
cvi/cva	Structural genes of colicin V operon (microcin ColV)	8.1	10.8	9.1	10.8
pic	Serine protease autotransporter	11.3	16.2	13.1	1.5
malX	Pathogenicity-associated island marker CFT073	14.5	27.0	19.2	0

[a] Primer references are cited in the study by Ewers et al. (4).
[b] Schierack et al. (10).
[c] *, differences between hemolytic isolates from diseased pigs and hemolytic isolates from clinically healthy pigs are statistically significant ($P < 0.05$). NT, not tested.
[d] This study.
[e] ETEC, enterotoxigenic *E. coli*.

eVAGs (Table 1; Fig. 2). All isolates carried the gene *ompA*. Two genes that were absent from all isolates were *iha* and *sat*. Only *tsh* and *traT* were found significantly more often in isolates from diseased piglets ($P < 0.05$). Genes *hra*, *chuA*, *fyuA*, *iroN*, *irp2*, *astA*, and *ibeA* were present significantly more often in isolates from healthy piglets ($P < 0.05$).

Hemolytic isolates belonged to EcoR groups A (59 isolates), B1 (14 isolates), B2 (18 isolates), and D (8 isolates). Isolates belonging to EcoR group B2 carried significantly more eVAGs (median, 18; minimum, 15; maximum, 22) than group D (median, 11; minimum, 7; maximum, 16), B1 (median, 9.5; minimum, 6; maximum, 18), and A (median, 7; minimum, 5; maximum, 15) members ($P < 0.001$) (see Table S2 in the supplemental material). Genes *sit* (chromosomal), *neuC*, *ibeA*, *gimB*, and *tia* were exclusively found in EcoR group B2 isolates. Almost all EcoR group B2 members carried genes *hra*, *papC*, *fyuA*, *malX*, *iroN*, *kpsMTII*, *sfa/focCD*, *irp2*, *sit* (chromosomal), *traT*, *astA*, *vat*, *hlyA*, and *cnf1/2* (Table S2).

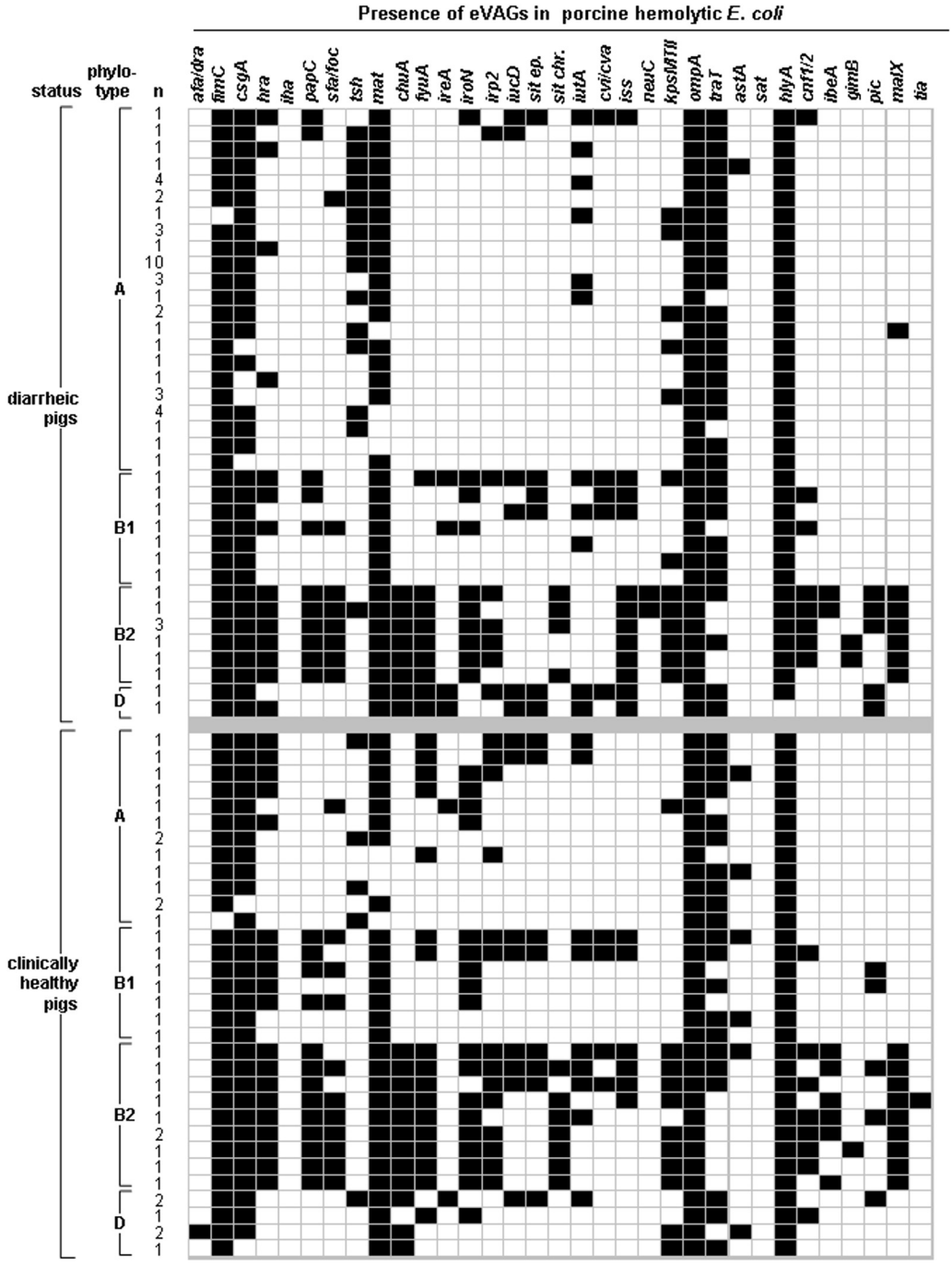

FIG. 2. Presence or absences of eVAGs in 99 porcine intestinal hemolytic *E. coli* isolates in this study. *n*, number of isolates per pattern. Solid squares indicate the presence of the respective gene, and open squares indicate its absence.

As hypothesized, a considerable proportion of porcine intestinal hemolytic *E. coli* isolates lacking iVAGs carried a broad variety of eVAGs. We identified a number of eVAGs that have not been reported for porcine *E. coli* isolates so far. Among these were genes coding for adhesins (*hra*, *tsh*, and *mat*), iron acquisition (*ireA*), protectins (*neuC*), and an invasion-related factor (*gimB*). Genes *iha* and *sat*, which are common in extraintestinal pathogenic *E. coli* (ExPEC) isolates were not detected, and only *iha* was previously reported to be present in intestinal *E. coli* from pigs (2, 13). In conclusion, almost all eVAGs can also be found in porcine intestinal *E. coli*.

A comparison between porcine intestinal hemolytic isolates without iVAGs of the present study with previously published intestinal porcine nonhemolytic isolates (10) showed that a broad variety of eVAGs were present in both groups (Table 1), but hemolytic isolates carried higher numbers of such VAGs. In line with other studies, *E. coli* isolates affiliated with EcoR

group B2 carried more eVAGs, in particular iron acquisition genes, than EcoR A, B1, or D isolates (5, 7).

In general, we detected more hemolytic isolates without iVAGs (82.2%) than with iVAGs (17.8%) in clinically healthy pigs. In contrast, a lower number of hemolytic isolates without iVAGs (25.0%) than with iVAGs (75.0%) were isolated from diarrheic pigs. Further studies should prove the role of hemolytic isolates without iVAGs in healthy and diseased pigs.

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