

**Original Article****Open Access****The role of *Escherichia coli* in the etiology of piglet diarrhea in selected pig producing districts of central Uganda***¹Obala, T., ²Arojjo, S. O., ¹Afayoa, M., ¹Ikwap, K., and ¹Erume, J.¹College of Veterinary Medicine, Animal Resources and Biosecurity, Makerere University,
P. O. Box 7062, Kampala, Uganda²College of Humanities and Social Sciences, Makerere University, P. O. Box 7062, Kampala, Uganda*Correspondence to obala.tonny14@gmail.com; +256773884308**Abstract:****Background:** Pig production in Uganda is highly constrained by rampant piglet mortalities with diarrhea being a key feature. The present study was conducted to determine possible involvement of *Escherichia coli* (*E. coli*) as agents of diarrhea in piglets and elucidate the factors for their spread and virulence, towards development of mitigation strategies in the smallholder pig value chains in Uganda.**Methodology:** This was a cross-sectional study carried out from January to August 2020 on pre- and post-weaned piglets from households in Kayunga and Mityana districts of Central Uganda, selected by snowballing method to redundancy. Data about herd management and risk factors for colibacillosis were collected from selected farmers in the two districts. A total of 179 faecal samples were collected from randomly selected neonatal and pre-weaning piglets for bacteriological isolation of *Escherichia coli*. Virulence (enterotoxin and fimbrial) genes from the isolates were detected by multiplex polymerase chain reaction (PCR) assay.**Results:** From the 179 faecal samples, a total of 158 (88.3%) *E. coli* isolates were obtained. Virulence gene markers were detected in 18.4% (29/158) of the isolates. Among the investigated genes encoding for enterotoxin production, ST_b was the most prevalent (16/158, 10.13%), followed by ST_a (12/158, 7.59%), while gene for LT was not detected. The gene coding for F4 adhesin was the only one detected while F18 adhesin was not detected from the isolates. On multiple logistic regression analysis, only tertiary educational level (OR=0.141; 95% CI=0.30-0.666; *p*=0.013) and infrequent use of antibiotics (OR=0.231, 95% CI=0.062-0.859; *p*=0.029) among the farmers, were the two factors significantly protective of the piglets from diarrhoea.**Conclusion:** This study reports a high prevalence of enterotoxin gene markers among *E. coli* isolates in piglets and revealed the potential role of these bacteria in the aetiology of piglet diarrhoea and mortalities in Uganda. Additionally, this study identified risk factors that can be useful in formulating treatment and control strategies of infection caused by these bacteria. Further studies are needed to identify more adhesins these *E. coli* isolates employ for intestinal colonization, a step that will help inform vaccine development.**Keywords:** Antibiotic resistance; *Escherichia coli*; piglet diarrhea; virulence factors; Uganda

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Le rôle d'*Escherichia coli* dans l'étiologie de la diarrhée des porcelets dans certains districts producteurs de porcs du centre de l'Ouganda*¹Obala, T., ²Arojjo, S. O., ¹Afayoa, M., ¹Ikwap, K., et ¹Erume, J.¹Collège de médecine vétérinaire, ressources animales et biosécurité, Université Makerere,
P. O. Box 7062, Kampala, Ouganda²Collège des sciences humaines et sociales, Université Makerere, P. O. Box 7062, Kampala, Ouganda*Correspondance à obala.tonny14@gmail.com; +256773884308**Abstrait:****Contexte:** La production porcine en Ouganda est fortement limitée par la mortalité généralisée des porcelets, la diarrhée étant une caractéristique clé. La présente étude a été menée pour déterminer l'implication possible

d'*Escherichia coli* (*E. coli*) en tant qu'agents de diarrhée chez les porcelets et élucider les facteurs de leur propagation et de leur virulence, vers le développement de stratégies d'atténuation dans les chaînes de valeur des petits producteurs de porcs en Ouganda.

Méthodologie: Il s'agit d'une étude transversale réalisée de janvier à août 2020 sur des porcelets pré- et post-sevrés issus de ménages des districts de Kayunga et Mityana du centre de l'Ouganda, sélectionnés par la méthode boule de neige jusqu'à la redondance. Les données sur la gestion du troupeau et les facteurs de risque de colibacillose ont été recueillies auprès d'éleveurs sélectionnés dans les deux districts. Au total, 179 échantillons de matières fécales ont été prélevés sur des porcelets néonataux et en pré-sevrage sélectionnés au hasard pour l'isolement bactériologique d'*Escherichia coli*. Les gènes de virulence (entérotoxine et fimbrial) des isolats ont été détectés par une amplification en chaîne par polymérase (PCR) multiplex.

Résultats: À partir des 179 échantillons de matières fécales, un total de 158 (88,3%) isolats d'*E. coli* ont été obtenus. Des marqueurs du gène de virulence ont été détectés dans 18,4% (29/158) des isolats. Parmi les gènes étudiés codant pour la production d'entérotoxines, ST_b était le plus répandu (16/158, 10,13%), suivi de ST_a (12/158, 7,59%), tandis que le gène de la LT n'a pas été détecté. Le gène codant pour l'adhésine F4 était le seul détecté alors que l'adhésine F18 n'a pas été détectée dans les isolats. Sur l'analyse de régression logistique multiple, seul le niveau d'enseignement supérieur (OR=0,141; IC à 95%=0,30-0,666; $p=0,013$) et l'utilisation peu fréquente d'antibiotiques (OR=0,231, IC à 95%=0,062-0,859; $p=0,029$) parmi les éleveurs, étaient les deux facteurs de protection significative des porcelets contre la diarrhée.

Conclusion: Cette étude rapporte une prévalence élevée de marqueurs génétiques d'entérotoxines parmi les isolats d'*E. coli* chez les porcelets et a révélé le rôle potentiel de ces bactéries dans l'étiologie de la diarrhée et de la mortalité des porcelets en Ouganda. De plus, cette étude a identifié des facteurs de risque qui peuvent être utiles dans la formulation de stratégies de traitement et de contrôle de l'infection causée par ces bactéries. D'autres études sont nécessaires pour identifier plus d'adhésines que ces isolats d'*E. coli* utilisent pour la colonisation intestinale, une étape qui aidera à éclairer le développement de vaccins.

Mots-clés: Résistance aux antibiotiques; *Escherichia coli*; diarrhée de porcelet; facteurs de virulence; Ouganda

Introduction:

Pork is one of the most consumed meat products worldwide (1) and the demand for pig meat is expected to increase with the growth in global population and economy. After China, the European Union (EU) is the second biggest producer of pig meat with a yearly production of 22.3 million tons (2). Therefore, maintenance of good health of pigs is very important. Uganda is among sub-Saharan Africa countries where pig production is increasing rapidly. The pig prolificacy is helping provide food security, however, it is constrained by low post-natal piglet survival (3). A study to quantify and classify pre-weaning causes of piglet mortality and how mortality relates to piggery management was conducted in peri-urban areas of Kampala, and out of 681 piglets born, 222 piglets died pre-weaning, representing 32.6% mortality (4). This is very high mortality considering that efficiency of piggery depends on the surviving litter size. In that study, infectious diseases were among the major causal factors reported. Moreover, during the smallholder pig value chain assessment in Uganda conducted by International Livestock Research Institute (ILRI) (3), among the major complaints' farmers put forward was rampant diarrhea. Similarly, in another study conducted in Gulu and Soroti, 38% of the pig keeping households reported diarrhea as a problem (5).

Globally, poor piglet survival is mainly due to *Escherichia coli*-induced diarrhea (6) or due to other bacterial pathogens (7). However, the causes of diarrhea/mortalities in the piglets

have not been determined in Central Uganda, the major pig-producing region of the country. *Escherichia coli* infections seriously affect pig productivity through diarrhea, growth retardation, high morbidity and mortality among diseased pigs (6). Two distinct diarrhea syndromes are majorly recognized; neonatal diarrhea in piglets from 1-7 days of life, and post-weaning diarrhea that manifests about a week after weaning (8). The objectives of this study are to establish the involvement of *E. coli* pathogens in diarrhea among Ugandan piglets and to characterize their virulence genes, which is aimed towards adoption/development of appropriate vaccines for application to prevent piglet diarrhea and related mortalities in the small holder pig value chains in Uganda.

Materials and method:

Study area and design

This was a cross-sectional study carried out from January to August 2020 on pre- and post-weaned piglets from selected households in Kayunga and Mityana districts of Central Uganda. The location of Mityana district is between 00 27N and 32 03E while the coordinates for Kayunga district are 01 00N and 32 52E.

Sample size determination

The sample size of households included in the study was arrived at using the Cochran formula; $n = Z\alpha^2 (P*Q)/E^2$ (9), where, "n" is the sample size and "P" is the proportion of households keeping pigs. In this study, "P" was taken

as 6%, which is the percentage of households rearing pigs in Kayunga (10) and "Q" is the proportion of households not keeping pigs (1-P). The "E" is the level of acceptable error and was taken to be 5% while the "Z α " is the 95% confidence interval taken to be 1.96. Therefore, total number of households keeping pigs' targeted for the study was 87.

Ethical consideration

Ethical approval was obtained from the Ethical Review Committee of the School of Biosecurity, Biotechnical and Laboratory Sciences, College of Veterinary Medicine, Animal Resources and Biosecurity, Makerere University. Pig farmers were informed about the research and their consent to participate in the study was obtained before start of data collection.

Sampling, administration of questionnaire and collection of faecal samples

There was no information available regarding the households' keeping pigs in Uganda, therefore identification of the households was done using the Snowballing method to redundancy (11,12). Pig keeping households were visited to administer questionnaire and collect stool samples from diarrheic and non-diarrheic piglets. The first farmer was identified by the help of the extension agents and subsequent farmers were identified by the help of fellow farmers. A questionnaire was administered to selected farmers after obtaining their consent verbally in order to ascertain the factors that may underlie the spread of *E. coli* diarrhea in their piggeries. The roles of various household members in relation to pig care and management were also assessed particularly in relation to their gender.

Subsequently faecal samples were collected randomly from 2 piglets in each litter and before any antibiotic treatment was given in case of diarrhea, to ensure optimum recovery of *E. coli*. During the sample collection, each piglet was scored as being diarrheic or not. The faecal samples from each piglet were collected per rectum using sterile swabs (BD, Loveton, Maryland, USA) and were immediately placed into 5 ml of sterile chilled peptone water medium. The samples were then transported on ice in a cool box to the Microbiology laboratory at the College of Veterinary Medicine, Animal Resources and Biosecurity, Makerere University, Uganda within 48 hrs of sampling for bacteriological culture and isolation.

Bacteriological isolation and identification

Each rectal swab was streaked out on sterile MacConkey agar (Condalab, Madrid, Spain) for the isolation of *E. coli*. Inoculated

plates were incubated aerobically at 37°C for 18-24 hr. Preliminary identification of isolates was based on colonial morphology on all the media used. Lactose-fermenting colonies on MacConkey agar were biochemically confirmed as *E. coli* using tryptophan broth for indole test, methyl red for MR/VP test and citrate agar for citrate utilization test. Biochemically confirmed *E. coli* were stored in Brain heart infusion broth (Oxoid Ltd, Hampshire, United Kingdom) with 20% glycerol in duplicate at -20°C pending DNA extraction.

Antibiotic susceptibility tests

Antimicrobial susceptibility testing of *E. coli* isolates was performed by the standard Kirby-Bauer disk diffusion method against selected antibiotics (Oxoid Ltd, Hampshire, United Kingdom) which includes; kanamycin (K 30 μ g), nalidixic acid (NA 30 μ g), tetracycline (TE 30 μ g), ampicillin (AMP 10 μ g), chloramphenicol (C 30 μ g), erythromycin (E 15 μ g), gentamicin (CN 10 μ g) and ciprofloxacin (CIP 5 μ g) on Mueller-Hinton agar (Oxoid Ltd, Hampshire, United Kingdom). The 2018 Clinical and Laboratory Standards Institute (CLSI) guidelines (13) were used for the interpretation and categorization of the test strains as sensitive (S), intermediate (I) or resistant (R). Control *E. coli* strain ATCC 25922 was also set along with positive isolates during susceptibility testing for each antibiotic used.

Extraction of DNA from *E. coli* isolates

DNA was extracted from each isolate using the boiling method (5). Briefly, the stored isolates were thawed thoroughly before vortexing to ensure uniformity of the bacterial cell suspension. One ml of the sample was transferred into a correspondingly labelled 1.5 ml Eppendorf tubes, and centrifuged at 15,000 \times g for 15 min in a microcentrifuge. The supernatant was discarded and remaining pellets were washed twice (by resuspending in 1 ml nuclease free water) followed by centrifugation at 15,000 \times g for 10 min in a microcentrifuge. The pellets were then re-suspended in 100 μ l of nuclease free water and boiled at 100°C for 10 min in a water bath. The tubes were thereafter frozen at -20°C for 10 min in a freezer and after thorough thawing, were centrifuged at 15,000 \times g for 10 min in a microcentrifuge. Finally, 50 μ l of the supernatant was transferred into correspondingly labelled Eppendorf tubes and stored at -20°C pending analysis by PCR.

Amplification of fimbrial and enterotoxin genes by multiplex PCR assay

Multiplex PCR assay was used to amplify the fragments of genes encoding enterotoxins

(ST_a, ST_b and LT) and fimbriae (F4 and F18) of each *E. coli* isolate. The primers used are as shown in Table 1. Each reaction consisted of 1× PCR buffer II, 3 mM MgCl₂, 200 μM each of dATP, dTTP, dCTP and dGTP and 1.5U of AmpliTaq Gold DNA polymerase (Applied Biosystems, ThermoFisher Scientific Corporation, Massachusetts, USA). The cycling conditions were set as follows; 95°C for 5 min, followed by 35 cycles of 94°C for 60 sec, 59°C for 60 sec and 72°C for 60 sec, followed by a final extension at 72°C for 5 min. DNA from *E. coli* strains 853/67; O149 (F4⁺, F6⁺, LT⁺, ST_a⁺, ST_b⁺ and EAST1⁺) (Uppsala, Sweden) and a blank sample without DNA were used as positive and negative controls, respectively.

Agarose gel electrophoresis

Eight microliters of each of the PCR amplicons were mixed with 2 μL of the loading buffer and resolved on 2% agarose gel in 1×TBE buffer at 125 V for 45 min using mupid-exu submarine electrophoresis system. The gels were stained by ethidium bromide, visualized by UV-transillumination and gel pictures captured by Smartphone camera.

Statistical analysis

Data obtained from the questionnaires were entered into a Microsoft Excel spreadsheet

and descriptive statistics was performed. A univariate analysis using Chi-square test or Fisher's exact test was used to analyze the laboratory data and also test the associations between the independent variables and the outcome variable. Variables with $p \leq 0.25$ were then tested for confounding by cross tabulation of two variables. If the variables were confounding ($p \leq 0.05$), only one was taken for multivariate logistic regression analysis based on biological plausibility. The model fitness was assessed by Hosmer and Lemeshow (HL) test.

Results:

Socio-demographics of the farmers involved in the study.

In total, samples were collected from 74 households; 35 and 39 in Mityana and Kayunga districts, respectively (Table 2). Male gender dominated pig farming in the both districts, with 19 (52.3%) and 28 (71.8%) from Mityana and Kayunga, respectively. Majority of farmers in Mityana were in age group of 31-40 years (34.3%), while in Kayunga, piggery was practiced mostly by farmers who were between 51-60 years of age (33.3%), which indicated that the youth were less frequently involved in piggery in the two districts (Table 3).

Table 1: PCR primers for amplifying specific DNA fragments of enterotoxin and fimbrial adhesin genes

Gene target	Primer sequence	Amplified product (bp)	Reference
LT	F - TAG AGA CCG GTA TTA CAG AAA TCT GA	282	(29)
	R - TCA TCC CGA ATT CTG TTA TAT ATG TC		
ST _a	F - GGG TTG GCA ATT TTT ATT CTG TA	183	(29)
	R - ATT ACA ACA AAG TTC ACA GCA GTA		
ST _b	F - ATG TAA ATA CCT ACA ACG GGT GAT	360	(29)
	R - TAT TTG GGC GCC AAA GCA TGC TCC		
F4	F - ATC GGT GGT AGT ATC ACT GC	601	(30)
	R - AAC CTG CGA CGT CAA CAA GA		
F18	F - GTG AAA AGA CTA GTG TTT ATT TC	510	(27)
	R - CTT GTA AGT AAC CGC GTA AGC		

F = Forward primer; R = Reverse primer; bp = base pair

Table 2: Number of households and sub counties selected in two districts of central Uganda

District	Sub-county	No of selected households
Mityana	Butayunja	12
	Kakindu	6
	Zigoti Town Council	8
	Mityana Municipality	9
Kayunga	Kayunga Town council	11
	Kangulumira	10
	Kitimbwa	8
	Kayunga	10

Table 3: Demographic characteristics of the household heads, pig owners, breeds and pig herd sizes in selected households in Mityana and Kayunga districts of Central Uganda

Demographic variables		Mityana n (%)	Kayunga n (%)	Total n (%)	p value
Gender	Male	19 (54.3)	28 (71.8)	47 (63.5)	0.118
	Female	16 (45.7)	11 (28.2)	27 (36.5)	
Age group (Years)	10-20	2 (5.7)	2 (5.1)	4 (5.4)	0.90
	21-30	1 (2.9)	4 (10.3)	5 (6.8)	
	31-40	12 (34.3)	4 (10.3)	16 (21.6)	
	41-50	9 (25.7)	8 (20.5)	17 (23.0)	
	51-60	5 (14.3)	13(33.3)	18 (24.3)	
	>61	6 (17.1)	8 (20.5)	14 (18.9)	
Education level	None	5 (14.3)	5 (12.8)	10 (13.5)	0.113
	Primary	14 (40.0)	9 (23.1)	23 (31.1)	
	Secondary	7 (20.0)	18 (46.2)	25 (33.8)	
	Tertiary	9 (25.7)	7 (17.9)	16 (21.6)	
Occupation	Farmer	18 (51.4)	18 (46.2)	36 (48.6)	0.438
	Civil Servant	2 (5.7)	4 (10.3)	6 (8.1)	
	Self employed	13 (37.1)	16 (41.0)	29 (39.2)	
	Student	2 (5.7)	0	2 (2.7)	
Ownership	Builder	0	1 (2.6)	1 (1.4)	0.016*
	Husband	9 (25.7)	18 (46.2)	27 (36.5)	
	Wife	17 (48.6)	15 (38.5)	32 (43.2)	
	Male child	2 (5.7)	5 (12.8)	7 (9.5)	
	Girl child	0	1(2.6)	1 (1.4)	
Breed of pigs	Family	7 (20.0)	0	7 (9.5)	0.01*
	Local	22 (62.9)	8 (20.5)	30 (40.5)	
	Exotic	8 (22.9)	23 (59.0)	31 (41.9)	
Pig herd size	Local & Exotic	5 (14.3)	8 (20.5)	13 (17.6)	0.153
	(1-10)	26 (74.3)	19 (48.7)	45 (60.8)	
	(11-20)	4 (11.4)	10 (25.6)	14 (18.9)	
	(21-30)	4 (11.4)	7 (17.9)	11 (14.9)	
	>30	1 (2.9)	3 (7.7)	4 (5.4)	
Pig Caretaker	House Wife	13 (37.1)	11 (28.2)	24 (32.4)	0.025*
	Husband	2 (5.7)	9 (23.1)	11 (14.9)	
	Male Child	4 (11.4)	4 (10.3)	8 (10.8)	
	Female Child	3 (8.6)	0	3 (4.1)	
	Hired Person	3 (8.6)	10 (25.6)	13 (17.6)	
	Family	10 (28.6)	5 (12.8)	15 (20.3)	

^an" denotes the number of selected households; * = statistically significant at $p < 0.05$

In Mityana, 14/35 (40%) farmers had primary level of education only, while in Kayunga, 18/39 (46.2%) farmers were educated up

to secondary level. These statistics show that 13.5% of all those involved in keeping pigs did not have formal education. The difference in the

education level of pig farmers in the two districts was however not statistically significant ($p=0.113$). However, 48.6% of those engaged in piggery in both districts were involved in farming as their major occupation. Wife owned pigs in 32 of the 74 households (43.2%) while in Mityana alone, 48.6% (17/35) of pigs were owned by wife. There was significant difference ($p=0.016$) in the ownership of pigs in both districts, with wife owning more pigs than husband. In both Kayunga and Mityana, housewives were most frequently involved in caring for the pigs (32.4%). Hired labourers were more frequently involved in pig care in Kayunga (25.6%) compared to Mityana (8.6%). On the other hand, husbands were less frequently involved in pig care (14.9%) (Table 3).

Fifty-nine percent of farmers in Kayunga reared exotic pig breeds, but Mityana farmers mostly reared local breeds (62.9%). In both districts, farmers were mostly involved in small scale pig rearing of about 1-10 per household. Additionally, 5.4% of farmers in this study were keeping more than 30 pigs per herd. However, the differences in herd size in both districts were not significant ($p > 0.05$).

***E. coli* strains isolated**

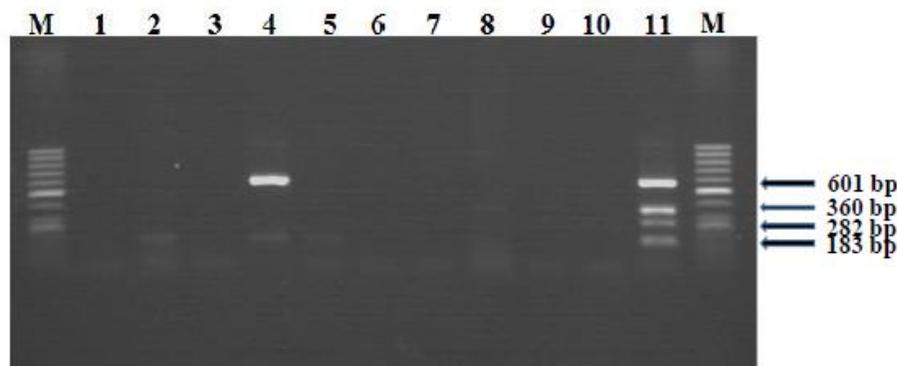
From the total of 179 faecal samples collected, 158 (88.3%) *E. coli* isolates were obtained following bacteriological culture. Majority of the *E. coli* isolates (85, 53.8%) were from Kayunga while 73 (46.2%) were from Mityana district (Table 4).

Virulence genes detected

Fig 1 shows a representative electrophoretic gel of virulence gene detection by PCR. Multiplex PCR assay revealed that 18.4% (29/158) of the isolates harbored virulence genes. Among the investigated genes coding for toxin production, ST_b was the most prevalent (16/158, 10.13%), followed by ST_a (12/158, 7.59%), while gene for LT was not detected. Gene for fimbriae was present in 0.63% (1/158) of the isolates. The F4 was the only fimbrial gene detected in this study while F18 was not detected in any of the isolates. The one *E. coli* strain carrying F4 gene also carried enterotoxin ST_a gene, and no fimbrial adhesin was detected in the rest of 157 (99.4%) *E. coli* isolates (Fig 2).

Table 4: Frequency of *Escherichia coli* isolation from the 179 faecal samples

Isolates	Mityana	Kayunga	Total (%)
<i>Escherichia coli</i> isolated	73	85	158 (88.3)
No <i>Escherichia coli</i> isolated	10	11	21 (11.7)
Total	83	96	179 (100)



Lanes M = Molecular weight standard (200 base pair ladder); Lanes 1 to 9 = 10 *Escherichia coli* isolates tested; Lanes 10 = Negative control; Lane 11 = Positive control. Top arrow indicates the position of migration expected for the amplified fragment produced by the amplification protocol for F4 (601 bp), followed by ST_b (360 bp), LT (282 bp) and ST_a (183 bp)

Fig 1: Gel electrophoresis of PCR products of fimbrial and enterotoxin genes of selected *Escherichia coli* isolates

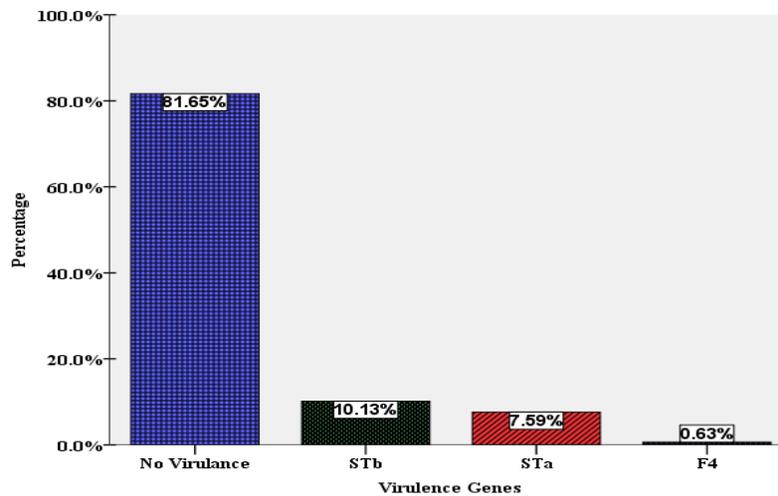


Fig 2: Frequency distribution of virulence genes in the 158 *Escherichia coli* Isolates

Table 5: Univariate analysis of statistically significant factors associated with piglet diarrhea

Factors	Diarrheic	Non-diarrheic	p value
Occupation	Farmer	23	0.027
	Civil Servant	6	
	Self-employed	13	
	Student	0	
	Builder	0	
Education level	None	7	0.020
	Primary	15	
	Secondary	8	
	Tertiary	12	
Breed of pigs kept	Local	21	0.029
	Exotic	12	
	Both	9	
District	Mityana	25	0.020
	Kayunga	17	
Frequent use of antibiotics	Yes	19	0.01
	No	23	

Risk factors associated with diarrhea in piglets

Sixteen independent variables; sex of household head, education level, age of household head, occupation, ownership of the farm, district, breed of pigs, breeding method, pig herd size, pigs keeping methods, house provided for neonate, pig care taker, source of water, weaning period, diarrhea common in the farm and common use of antibiotics, were analyzed as potential risk factors in the causation of diarrhea in piglets in the total of 74 farms in Mityana and Kayunga districts. Univariate analysis shows that occupation, education level, breed of pigs, district and frequent use of antibiotics were significantly associated ($p < 0.05$) with diarrhea in the piglets (Table 5). At multivariate analysis, two factors (education level and frequent use of antibiotics) were signifi-

cantly associated with diarrhea in the piglets (Table 6). Tertiary education (OR=0.141, 95% CI=0.30-0.666, $p=0.013$) and infrequent use of antibiotics (OR=0.231, 95% CI=0.062-0.859, $p=0.029$) among the farmers were the two factors significantly protective of the piglets from diarrhoea.

Results of antimicrobial susceptibility test

The antibiotic to which the *E. coli* isolates exhibited the most resistance in both districts was erythromycin (Fig 3), followed by tetracycline. In general, the *E. coli* isolates from Kayunga district showed higher resistance to the antibiotics compared to those from Mityana district (Table 7). The most effective antibiotic drug was kanamycin, followed by ciprofloxacin and gentamicin.

Table 6: Risk factors associated with piglet diarrhea in multivariate logistic regression model

Factors	Coefficient (B)	SE	OR	95% CI	p value
Education level	-1.960	0.793	0.141	0.30 - 0.666	0.013*
Breed of pigs	0.128	0.861	1.137	0.210 - 6.148	0.882
House provided for neonate	-0.195	0.625	0.823	0.242 - 2.802	0.756
Frequently use antibiotics	-1.466	0.670	0.231	0.062 - 0.859	0.029*
Constant	2.882	1.008	17.859	-	0.004

Hosmer and Lemeshow Test $p = 0.003$; Model = 0.257; * = statistically significant; OR = Odds Ratio; SE = Standard Error; CI = Confidence Interval

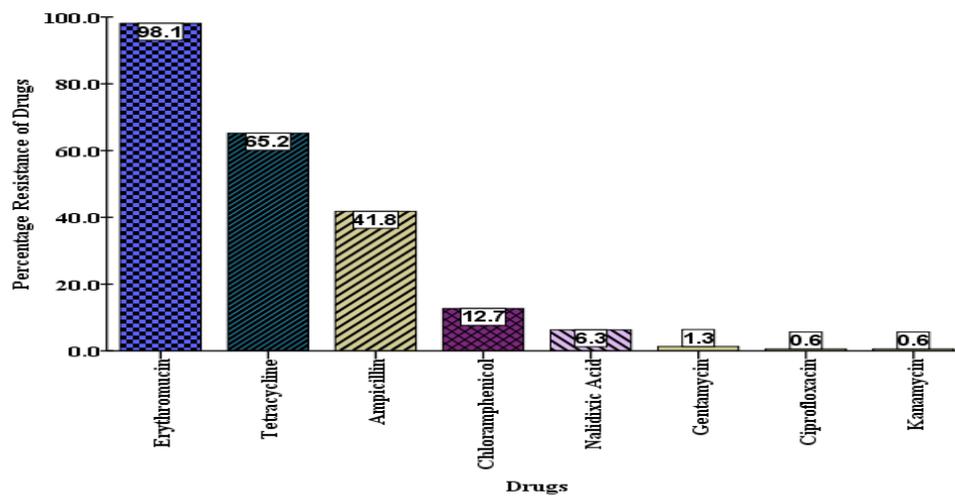


Fig 3: Antimicrobial resistance of 158 Escherichia coli isolates obtained from the piglets

Table 7: Comparison of resistance rates of different isolates between Mityana and Kayunga districts of Uganda

Antibiotics	Mityana n (%)	Kayunga n (%)	Total n (%)	p value
Kanamycin	0	1 (1.2)	1 (0.6)	1.000
Nalidixic acid	6 (8.3)	4 (4.7)	10 (6.3)	0.514
Tetracycline	38 (52.8)	65 (75.6)	103 (65.2)	0.004*
Ampicillin	28 (38.9)	38 (44.2)	66 (41.8)	0.521
Chloramphenicol	8 (11.1)	12 (14.0)	20 (12.7)	0.638
Erythromycin	69 (95.8)	85 (100)	154 (98.1)	0.092
Gentamycin	1 (1.4)	1 (1.2)	2 (1.3)	1.000
Ciprofloxacin	1 (1.4)	0	1 (0.6)	0.456

* = statistically significant at $p < 0.05$

Discussion:

This was the first study to elucidate the role of *E. coli* in the aetiology of piglet diarrhea as well as the risk factors and antimicrobial resistance of these pathogens in Central Uganda. The finding of 18.4% of the *E. coli* with virulence factors (fimbriae and/or enterotoxin genes) is significant since it confirms the possible involvement of these bacteria in the etiology of diarrhea and piglet mortalities in Uganda. In the present study, the only adhesin detected in *E. coli* was F4, which is in agreement with the previous study conducted in Northern and Eastern Uganda by Ikwap et al., (5) but differ from developed countries (14) where F4 was most predominant. In our study, F18 adhesin was not detected, however, F18 adhesin was recently reported in diarrheic weaners from large commercial farms in Central Uganda (15). Since F18 adhesin is associated with post weaning diarrhoea (PWD), the prevalence of PWD could be very low in weaners from small holder herds, since this condition is mainly related to intensive rearing systems with high infectious load, stress caused by early weaning, moving and mixing of pigs (5,16) Secondly, the non-detection of F18 could be due to the low number of diarrheic piglets tested in our study. Interestingly, no adhesins were detected in 99.4% of the *E. coli* isolates tested in this study, although we investigated only F4 and F18 fimbriae. There is the need to therefore conduct more research on other adhesins in order to determine the key adhesins used by these bacteria to colonize piglet intestines, which can subsequently lead to diarrhea. This step will enable identification of appropriate vaccines for disease prevention.

In our study, the prevalence of piglet diarrhea was 16.8%. This is similar to the finding of a study conducted in India by Vinodh-Kumar et al., (17) which reported a prevalence of 14.29%, although slightly higher than 10% rate reported in a similar study conducted in Spain by Mesonero-Escuredo et al., (18). Worldwide, *E. coli* expressing enterotoxins (ETEC) are well known to cause severe diarrhea often with high mortality rates in both neonatal and post-weaning piglets (19). The ETEC strains associated with piglet diarrhea normally produce LT, ST_a or ST_b toxins or their combinations (20). In this present study, ST_a and ST_b were the only toxin genes detected, and in agreement with previous studies that reported high prevalence of ST_b in *E. coli* isolates from neonatal and weaned piglets (21,22), the most prevalent enterotoxin detected in *E. coli* from piglets in our study was ST_b (10.13%). However, the gene for LT was not detected in our isolates, suggesting

that *E. coli* harboring genes encoding for this toxin are not yet widespread. The absence of the gene for LT in all of the *E. coli* isolates in our study is in agreement with previous study in Northern and Eastern Uganda by Ikwap et al., (5) where none of the LT gene was detected. This suggests that ETEC diarrhea in Central Uganda could be largely contributed by ST_b and ST_a in neonatal and post-weaning piglets. In order to cause colibacillosis, an ETEC strain needs to simultaneously carry at least one fimbria and one toxin gene (23). Considering this criterion, less than 1% of the isolates in our present study could be classified as ETEC since there was only one ST_a/F4 combination detected in this study. The other isolated strains might have been non-pathogenic or belonged to other pathotypes such as EPEC and the fimbrial type F5, F6 or F41, which were not investigated in this study.

Two factors (tertiary education level and infrequent use of antibiotics) were significantly protective of the pigs from diarrhea from our study analysis. This may probably indicate that highly educated farmers (with tertiary level education) consider hygiene and other disease control measures more importantly than less educated farmers (with secondary level education) and therefore adhere to the required processes to control and prevent disease. However, if a farmer with formal education lacks training and experience in animal husbandry, it can lead to flaw in pig production as knowledge uptake itself does not necessarily guarantee an improvement in the everyday practices (24). Pig farmers in the study area were also involved in other farming activities, self-employed or were civil servants. This picture gives the impression that pig farming alone does not make farmers to make a living within the study area.

Infrequent use of antibiotics was significantly associated with lower frequency of diarrhea in the piglet probably because of the adherence to disease control measures such as environment hygiene and proper feeding. Farmers who frequently use antibiotics tend to assume that these drugs are a panacea to proper rearing of pigs, yet not all antibiotics are effective against bacteria pathogens. For treatment to be effective against disease-causing bacteria, antibiotics must reach the site of infection at sufficient concentrations and for sufficient amount of time. Inappropriate use of these antibiotics may not only promote antimicrobial resistance but also aggravate the disease. Furthermore, commensal beneficial bacteria provide a natural barrier and defense system against pathogenic bacteria, treatment with antibiotics results in killing of these beneficial bacteria, thereby rem-

oving the natural defense system of the animals and making them more vulnerable to disease.

Antimicrobial resistance profiles of *E. coli* isolates in the present study revealed high resistance to erythromycin and tetracycline. This is highly concerning considering that the tetracyclines are the most frequently used antibiotics for treatment of infections in animals and poultry in Uganda, compared to other antimicrobial agents (25). Therefore, the high resistance levels in our study might be the result of widespread and frequent use of tetracycline to treat colibacillosis in the field. Interestingly one of the risk factors detected associated with piglet diarrhea was frequent use of antimicrobial agents against infections in the pig herds. This is attributable to probable emergence of antimicrobial resistance, hence the inability to control infections using the commonly available antimicrobials in these households.

Resistance to ampicillin, a commonly prescribed antibiotic for the treatment of colibacillosis, was relatively high with overall resistance of 41.8% in this study. A study done in Korea by Kyung-Hyo et al., (26) showed higher resistance of 84.9%. Overuse of ampicillin in Uganda to treat piglets against diseases such as clostridiosis and colibacillosis has probably led to such high degree of resistance of *E. coli* to this drug. Withdrawing such antibiotic from use by making it unavailable in the market and/or restricting its use may allow it to recover its potency, and may then subsequently be re-introduced, through a process of antibiotic cycling.

Majority of *E. coli* isolates in this study were susceptible to kanamycin, gentamicin, ciprofloxacin, nalidixic acid, and chloramphenicol. These findings are similar to that of a previous study on *Salmonella* spp in piglets and weaners from Northern and Eastern Uganda (27). Apart from gentamicin and kanamycin, the other three antimicrobials (chloramphenicol, ciprofloxacin and nalidixic acid) are mainly used in humans in Uganda. Furthermore, some of these antibiotics are very expensive and therefore not commonly used. Possibly this could be the reason for the high susceptibility of the piglet derived isolates of *E. coli* to these drugs in the present study. However, high levels of resistance to gentamicin were reported among *E. coli* isolated from diseased pigs in Belgium, Poland and Spain with rates of 46%, 45%, and 20% respectively (28). There is therefore the need for continuous monitoring, restrictions and judicious use of antimicrobials to ensure the future availability of effective antimicrobial drugs for use in both humans and veterinary medicine in Uganda.

Conclusion:

This study reports a high prevalence of enterotoxin gene markers in *E. coli* in tested piglets therefore highlighting the potential role of these bacteria in the aetiology of piglet diarrhea and mortalities in Uganda. Additionally, this study identified risk factors that can be useful in formulating treatment and control strategies for infections caused by these bacteria. Since no adhesins were identified in most of the *E. coli* isolates, further studies are needed to identify the adhesins these *E. coli* isolates employ for intestinal colonization, a step that will help inform vaccine development in Uganda settings.

Conflict of interest:

Authors declare no conflict of interest.

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References:

1. Food and Agriculture Organization (FAO). World Food and Agriculture – Statistical Pocketbook, Rome, 2018.
2. United States Department of Agriculture, Foreign Agricultural Service. Livestock and Poultry: World Markets and Trade, 2014.
3. Ouma, E., Dione, M., Lule, P., Roesel, K., and Pezo, D. Characterization of smallholder pig production systems in Uganda: constraints and opportunities for engaging with market systems. *Livestock Research for Rural Development* 26(3): Article #56. <http://www.lrrd.org/lrrd26/3/ouma26056.htm>
4. Biryomumaisho, S., and Ogalo, R. A study of pre-weaning piglet mortality in selected farms in the peri-urban areas of Kampala, Uganda. *Afri J Anim Biomed Sci.* 2007; 2 (1): 1-5.
5. Ikwap, K., Larsson, J., and Jacobson, M., et al. Prevalence of adhesin and toxin genes in *E. coli* strains isolated from diarrheic and non-diarrheic pigs from smallholder herds in northern and eastern Uganda. *BMC Microbiol.* 2016; 16: 178. <https://doi.org/10.1186/s12866-016-0796-2>.
6. Verdonck, F., Cox, E., van Gog, K., et al. Different

- kinetic of antibody responses following infection of newly weaned pigs with an F4 enterotoxigenic *Escherichia coli* strain or an F18 verotoxigenic *Escherichia coli* strain. Vaccine. 2002; 20 (23-24): 2995-3004.
7. Alsop, J. E. An outbreak of salmonellosis in a swine finishing barn. J Swine Hlth Prod. 2005; 13 (5): 265-268.
 8. Amezcua, R., Friendship, R. M., Dewey, C. E., Gyles, C., and Fairbrother, J. M. Presentation of post-weaning *Escherichia coli* diarrhea in southern Ontario, prevalence of hemolytic *E. coli* serogroups involved, and their antimicrobial resistance patterns. Can J Vet Res. 2002; 66 (2): 73 - 78.
 9. Pfeiffer, D. U. Veterinary Epidemiology – An Introduction. Epidemiology Division, Department of Veterinary Clinical Sciences, The Royal Veterinary College, University of London, Hawkshead Lane, North Mymms, Hertfordshire AL9 7TA, United Kingdom, 2002.
 10. Uganda Bureau of Statistics (UBOS). A report of Agricultural module, piggy-backed onto the Population and Housing Census (PHC). 2004
 11. Kagira, M. J., Kanyari, W. N. P., Maingi, N., Githigia, M. S., Ng'ang'a, J. C., and Karuga, W. J. Characteristics of the smallholder free-range pig production system in western Kenya. Trop Anim Hlth Prod. 2010; 42 (5): 865-873.
 12. Pondja, A., Neves, L., Mlangwa, J., et al. Prevalence and risk factors of porcine cysticercosis in Angonia district, Mozambique. PLoS Negl Trop Dis. 2010; 4 (2): e594
 13. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Disk Susceptibility Tests. 13th edn. CLSI standard M02. Wayne, PA, 2018
 14. Zutic, J., Asanin, J., Mistic, D., et al. Isolation of ETEC strains from piglets with diarrhea in the neonatal period and their typization based on somatic and fimbrial antigens. Acta Vet Brno. 2010; 60 (5-6): 497-506.
 15. Okello, E., Moonens, K., Erume, J., and De Greve, H. Enterotoxigenic *Escherichia coli* strains are highly prevalent in Ugandan piggeries but disease outbreaks are masked by antibiotic prophylaxis. Trop Anim Hlth Prod. 2015; 47 (1): 117-122.
 16. Kim, Y. J., Kim, J. H., Hur, J., and Lee, J. H. Isolation of *Escherichia coli* from piglets in South Korea with diarrhea and characteristics of the virulence genes. Can J Vet Res. 2010; 74 (1): 59-64.
 17. VinodhKumar, O. R., Singh, B. R., Sinha, D. K., et al. Risk factor analysis, antimicrobial resistance and pathotyping of *Escherichia coli* associated with pre- and post-weaning piglet diarrhoea in organised farms, India. Epidemiol Infect. 2019 147: e174.
 18. Mesonero-Escuredo, S., Strutzberg-Minder, K., Casanovas, C., et al. Viral and bacterial investigations on the aetiology of recurrent pig neonatal diarrhoea cases in Spain. Porc Hlth Manag. 2018;4: 5.
<https://doi.org/10.1186/s40813-018-0083-8>
 19. Andrea, L. Swine enteric colibacillosis: diagnosis, therapy and antimicrobial resistance. Porcine Hlth Manag. 2017; 3: 16.
<https://doi.org/10.1186/s40813-017-0063-4>.
 20. Fairbrother, J., Nadeau, E., and Gyles, C. *Escherichia coli* in postweaning diarrhea in pigs: An update on bacteria types, pathogenesis, and prevention strategies. Animal Hlth Res Rev. 2005; 6 (1): 17-39.
 21. Costa, M. M., Drescher, G., Maboni, F., et al. Virulence factors, antimicrobial resistance, and plasmid content of *Escherichia coli* isolated in swine commercial farms. Arq Bras Med Vet Zootec. 2010; 62 (1): 30-36
 22. Moredo, F., Pineyro, P., Márquez, G., and Sanz, M. Enterotoxigenic *Escherichia coli* subclinical infection in pigs: Bacteriological and genotypic characterization and antimicrobial resistance profiles. Food borne Pathog Dis 2015; 12 (8): 704-711.
 23. Francis, D. H. Enterotoxigenic *Escherichia coli* infection in pigs and its diagnosis. J Swine Hlth Prod. 2002; 10 (4): 171-175.
 24. Ngowi, H. A., Mkupasi, E. M., Lekule, F. P., Willingham, A. L., and Thamsborg, S. M. Impact of farmer education on their knowledge, attitudes, and practices in southern Tanzania: a case for *Taenia solium* control. Livestock Res Rural Dev. 2011; 23: 2
 25. Susan, N., Miriam, K., Christine, N., Laurie, D. W., Clare, I. R. C., and Sarah, G. S. Use of antibiotics to treat humans and animals in Uganda: a cross-sectional survey of households and farmers in rural, urban and peri-urban settings. JAC Antimicrob Resist. 2020; 2 (4): dlaa082.
doi: 10.1093/jacamr/dlaa082
 26. Kyung-Hyo, D., Jae-Won, B., and Wan-Kyu, L. Antimicrobial Resistance Profiles of *Escherichia coli* from Diarrheic Weaned Piglets after the Ban on Antibiotic Growth Promoters in Feed. Antibiotics. 2020; 9 (11): 755.
<https://doi.org/10.3390/antibiotics9110755>
 27. Ikwap, K., Erume, J., Owiny, D. O., et al. Salmonella species in piglets and weaners from Uganda: prevalence, antimicrobial resistance and herd-level risk factors. Prev Vet Med. 2014; 115 (1-2): 39-47.
 28. Aarestrup, F. M., Oliver Duran, C., and Burch, D.G. Antimicrobial resistance in swine production. Anim Hlth Res Rev. 2008; 9 (2):135-48.
 29. Nguyen, T. L., Truong, H. T., and Nguyen, V. G. Determining virulence factors of *Escherichia coli* strains isolated from diarrhea piglets by using PCR method. J Sci Dev. 2009; 7 (2): 187 - 191.
 30. Ojeniyi, B., Ahrens, P., and Meyling, A. Detection of fimbrial and toxin genes in *Escherichia coli* and their prevalence in piglets with diarrhoea. The application of colony hybridization assay, polymerase chain reaction and phenotypic assays. J Vet Med B. 1994; 41 (1-10): 49-59