SAMPLING CONSIDERATIONS FOR HERD-LEVEL MEASUREMENT OF FAECAL *ESCHERICHIA COLI* ANTIMICROBIAL RESISTANCE IN DIFFERENT TYPES OF CALVES, PIGS AND TURKEYS PRODUCTION

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Background

Even if antimicrobial resistance discover was concomitant with the beginning of antimicrobial agents use, its importance in public health is growing during the last years (Heurtin-Le Corre et al., 1999). In 1999, the European Scientific Conference "The use of antibiotics in animals – Ensuring the protection of public health" insisted about the harmonization of national antimicrobial resistance monitoring and surveillance programmes in animals and animals derived foods. It was considered that *Escherichia coli* constitute an indicator bacteria for antimicrobial resistance. This resistance is of concern because *Escherichia coli* can be pathogenic to animals and humans (Hartl and Dykhuizen, 1984; Phillips et al., 1988) and they also can be reservoirs of antimicrobial genes to be transferred to other Gram-negative bacteria (Hunter et al., 1992). Foods are often contaminated with antimicrobial resistant *Escherichia coli* and represent an important source of these organisms for humans (Corpet, 1993; Feinman, 1998). Animal products (such as pork, veal and poultry) often harbour numerous antimicrobial resistant *Escherichia coli* (DANMAP, 2000). Food animals may have a high prevalence of antimicrobial resistant *Escherichia coli* in their fecal flora (Dunlop, 1998), and because fecal contamination of carcasses at slaughter is virtually inevitable, these organisms and their resistance may be transmitted to humans if these foods are improperly cooked.

Objectives

The purpose of this investigation was to estimate the abundance and patterns of antimicrobial resistance among *Escherichia coli* in the feces of calves, pigs and turkeys in herds with different exposition to antimicrobial agents. The first part of the study, presented here, concerns only two herds by production. It is a preliminarily phase for a larger second part done with 24 herds by production. Finally, the aim of this study is to state about the influence of the production system on the antimicrobial resistance abundance, to establish a reference for risk analysis of antimicrobial resistance and to give some useful methodological elements for antimicrobial resistance supervision.

Methods

Two herds by production were selected, with respectively high and low (or no) level of antimicrobial use. In each of these six herds, four samples are taken. The two first samples (A and B) are composed by 30 fecal swabs done the week before slaughter. The third sample (C) is an environmental sample done with gauze tampon. The last sample (D), as A and B, is made up of 30 fecal swabs realized before slaughter, but done later, on another batch. For each sample, antimicrobial susceptibility of 60 *Escherichia coli*, isolated on Mac Conkey agar after an incubation at 37°C for 24h, is tested against 16 antimicrobial agents by disk diffusion : Amoxicillin (AMO), Amoxicillin + Clavulanic acid (AMC), Cefalexin (CEF), Ceftiofur (EXC), Neomycin (NEO), Gentamicin (GEN), Apramycin (APR), Spectinomycin (SPE), Streptomycin (STR), Tetracyclin (TET), Chloramphenicol (CHL), Sulfamides (SUL), Trimethoprim (TMP), Nalidixic acid (NAL), Flumequin (FLU), Enrofloxacin (ENR).

Results and discussion

Antimicrobial resistance patterns were determined for 1440 colonies of *Escherichia coli* from the 24 pooled fecal samples (60 by sample). Table 1 sums up the percentages of non-susceptible (intermediate + resistant) colonies against each antimicrobial agent for each sample.
There is no resistance against Ceftiofur in all herds. Some resistances are very exceptional, Cefalexin resistance is present in only two samples in the turkeys and calves herds with high exposure to antimicrobial agents, Gentamicin resistance is found in calves herds and Apramycin resistant *Escherichia coli* are found only in the calve herd with high exposure. Neomycin presents low levels of resistance except in the calves herds. Amoxicillin resistance is not frequent in the swine herds, but it can reach very high levels in turkeys and calves herds, especially with high exposure to antibiotics, Amoxicillin + Clavulanic acid resistance is found when there is an high level in Amoxicillin resistance. Spectinomycin resistance is present in pigs and calves herds but not in turkeys ones. Quinolones and Chloramphenicol resistances are only a background in swine herds, they present low levels in calves and turkeys herds with low exposure but very high levels in the others herds. Four resistances are always present and have the higher levels in all herds (Streptomycin, Tetracycline, Sulfamides, Trimethoprim). Tetracyclin resistance is by far the most frequently found.
Sometimes there are high differences between the samples done in the same farm. Between A, B and C samples, the environmental (C) sample seems to be the less pertinent, it is especially the case in the calves herd with low exposure to antibiotics. The samples A, B and D present generally the same types of resistances, but with variable levels. Even between two similar samples (A and B) the difference of prevalence can be important, up to more than 50%. Those differences are explained by a different majority phenotype present in each sample. For example, in the swine herd with high exposure to antibiotics, the majority phenotype found in A sample is "SPE-STR-TET" (36 colonies/60) when in B sample it is "TET-SUL-TMP" (35 colonies/60).
The number of resistances for the same bacteria are presented in Table 2. No resistance is the most frequent phenotype, but there are large variations between herds and particularly levels of exposure to antimicrobial agents. No resistance is more common in the herds with a low exposure to antimicrobial agents when multiresistance is more present in herds with high exposure, especially calves herds, with up to 13 resistances for the same bacteria.

Conclusion

This methodological preliminary phase shows resistance-levels differences between species and production systems and justifies the continuation of this study on a larger number of farms. Fecal samples seem to be more pertinent than environmental ones and are preferably

done. Nevertheless, results of prevalence of antimicrobial resistance may present variations between similar samples. This source of variability means that results of resistance prevalence must be interpreted very carefully. Therefore, the second phase of this study will be done in 24 herds by production, with two samples by farms, at the beginning and at the end of the breeding time, and, for each sample, the determination of antimicrobial susceptibility on 30 *Escherichia coli*.

Pertinent literature

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Table 1. Percentages of non-susceptible *Escherichia coli* colonies for faecal and environmental samples done in pigs, turkeys and calves herds with low or high exposure to antimicrobial agents.

Specie	Level	Sample	AMO	AMC	CEF	EXC	NEO	GEN	APR	SPE	STR	TET	CHL	SUL	TMP	NAL	FLU	ENR
Pigs	Low	A	3	0	0	0	0	0	0	33	45	87	3	37	8	2	2	2
		B	7	0	0	0	5	0	0	12	40	58	8	18	10	0	0	0
		C	13	0	0	0	0	0	0	18	40	72	22	30	10	0	0	0
		D	3	0	0	0	0	0	0	12	38	60	3	17	7	0	0	0
	High	A	5	0	0	0	0	0	0	62	93	98	0	25	8	2	0	0
		B	2	0	0	0	0	0	0	23	30	88	0	73	58	0	0	0
		C	5	0	0	0	2	0	0	23	92	97	3	17	10	2	2	2
		D	0	0	0	0	0	0	0	72	73	100	0	73	70	0	0	0
Turkeys	Low	A	32	0	0	0	13	0	0	0	33	50	3	27	23	7	7	0
		B	23	0	0	0	13	0	0	0	22	53	2	23	23	3	3	0
		C	3	0	0	0	0	0	0	0	20	50	2	10	15	2	2	0
		D	30	0	0	0	5	0	0	0	42	52	2	35	35	13	0	0
	High	A	70	27	7	0	2	0	0	0	67	98	33	73	68	95	83	37
		B	70	2	0	0	0	0	0	0	60	100	40	80	72	47	47	7
		C	97	0	0	0	5	0	0	0	87	100	80	88	83	100	100	13
		D	20	0	0	0	0	0	0	0	37	100	17	57	57	17	17	0
Calves	Low	A	50	22	0	0	53	2	0	37	80	58	28	77	25	0	0	0
		B	38	10	0	0	20	0	0	30	47	37	23	40	12	0	0	0
		C	3	0	0	0	0	0	0	0	3	3	0	3	0	0	0	0
		D	97	33	0	0	97	0	0	18	98	98	15	98	17	10	10	0
	High	A	100	8	0	0	72	30	25	12	98	98	70	100	52	87	65	25
		B	95	18	2	0	78	38	32	17	97	98	55	97	48	80	50	35
		C	100	40	0	0	82	10	3	18	97	100	95	98	20	95	93	60
		D	100	7	0	0	68	28	3	28	83	100	90	97	93	97	93	73

Table 2. Distribution of the patterns of *Escherichia coli* resistance in pigs, turkeys and calves herds with low or high exposure to antimicrobial agents according to the number of resistances supported by bacteria.

Nb. resistances		0	1	2	3	4	5	6	7	8	9	10	11	12	13	Total	Nb. phenotypes
Pigs	Low	64	66	43	15	30	14	8	-	-	-	-	-	-	-	240	40
	High	10	17	41	111	14	46	-	-	1	-	-	-	-	-	240	16
Turkeys	Low	100	54	14	26	3	23	20	-	-	-	-	-	-	-	240	29
	High	-	27	14	27	23	15	20	9	89	5	10	1	-	-	240	43
Calves	Low	103	-	-	6	30	36	27	8	30	-	-	-	-	-	240	24
	High	1	-	-	-	1	12	15	1	35	94	54	20	5	2	240	50