



**HAL**  
open science

## **In vivo transfer of an incFIB plasmid harbouring a class 1 integron with gene cassettes**

Alieda van Essen-Zandbergen, Hilde Smith, Kees Veldman, Dik Mevius

### ► **To cite this version:**

Alieda van Essen-Zandbergen, Hilde Smith, Kees Veldman, Dik Mevius. In vivo transfer of an incFIB plasmid harbouring a class 1 integron with gene cassettes. *Veterinary Microbiology*, 2009, 137 (3-4), pp.402. 10.1016/j.vetmic.2009.02.004 . hal-00485543

**HAL Id: hal-00485543**

**<https://hal.science/hal-00485543>**

Submitted on 21 May 2010

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

## Accepted Manuscript

Title: In vivo transfer of an incFIB plasmid harbouring a class 1 integron with gene cassettes *dfrA1-aadA1*

Authors: Alieda van Essen-Zandbergen, Hilde Smith, Kees Veldman, Dik Mevius



PII: S0378-1135(09)00079-0  
DOI: doi:10.1016/j.vetmic.2009.02.004  
Reference: VETMIC 4358

To appear in: *VETMIC*

Received date: 30-9-2008  
Revised date: 29-1-2009  
Accepted date: 3-2-2009

Please cite this article as: van Essen-Zandbergen, A., Smith, H., Veldman, K., Mevius, D., In vivo transfer of an incFIB plasmid harbouring a class 1 integron with gene cassettes *dfrA1-aadA1*, *Veterinary Microbiology* (2008), doi:10.1016/j.vetmic.2009.02.004

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1 **In vivo transfer of an IncFIB plasmid harbouring a class 1 integron with gene**  
2 **cassettes *dfrA1-aadA1***

3  
4 **Alieda van Essen-Zandbergen<sup>1</sup>, Hilde Smith<sup>1</sup>, Kees Veldman<sup>1</sup> and Dik Mevius<sup>1,2</sup>**

5  
6 <sup>1</sup>Central Veterinary Institute (CVI) of Wageningen UR, PO box 2004, 8203 AA Lelystad, The  
7 Netherlands.

8 <sup>2</sup> Department of Infectious Diseases & Immunology, Faculty of Veterinary Medicine, Utrecht University,  
9 The Netherlands

10  
11 \*Corresponding author. Tel: +31320238800; Fax: +31320239153; E-mail: [alieda.vanessen@wur.nl](mailto:alieda.vanessen@wur.nl)

12  
13 **Abstract**

14 Transfer of resistance genes from bacteria from food producing animals to human pathogens is a  
15 potential risk to human health. The aim of this study was to determine *in vivo* transfer of a plasmid  
16 harbouring a class 1 integron containing gene cassettes *dfrA1-aadA1* from *Salmonella* to *E. coli* and  
17 the influence of the use of antimicrobials on this transfer. Thirty four-days-old SPF chickens colonized  
18 with *E. coli* K12 were divided into 3 groups of 10 animals each, and placed in separate isolators.  
19 Eleven days after inoculation with *E. coli* K12 the chickens were inoculated orally with 10<sup>4</sup> CFU of *S.*  
20 *enterica* spp. *enterica* serovar Typhimurium containing a plasmid harbouring a class 1 integron with  
21 gene cassettes *dfrA1-aadA1*. Two days after the administration of *S. Typhimurium* 1 group was  
22 treated orally with doxycycline, 1 group orally with trimethoprim/sulphamethoxazole and 1 group  
23 remained untreated (control group). *E. coli* K12, *S. Typhimurium* and the transconjugants were  
24 isolated from cloacal samples on selective MacConkey agar plates. Transfer of the plasmid was  
25 confirmed by plasmid characterization, PCR, PFGE and hybridization. Plasmid mediated transfer of a  
26 class 1 integron was observed almost immediately after inoculation with *S. Typhimurium*. Treatment of  
27 the chickens with antibiotics had neither a positive nor a negative effect on the transfer rates. In  
28 addition to the resistance genes located on the integron, resistance genes encoding for tetracycline  
29 and amoxicillin resistance transferred from the donor strain as well. The resistance genes and the  
30 integron were located on a 130 kb sized IncFIB plasmid. Our data demonstrate *in vivo* transfer of an

31 IncFIB plasmid harbouring a class 1 integron containing gene cassettes *dfrA1-aadA1* from *Salmonella*  
32 to *E. coli*, with or without selective pressure of antibiotics in chickens.

33

## 34 1. Introduction

35 Antimicrobial resistance and, in particular, multidrug resistance (MDR) is an increasing  
36 problem worldwide. Exposure to MDR bacteria via the food chain is considered a potential risk to  
37 human health either by food borne infections with resistant pathogens or by horizontal transfer of  
38 resistance genes from bacteria from food producing animals to human pathogens (Box et al., 2005;  
39 Leverstein-van Hall et al., 2003; Poppe et al., 2005). Integrons are genetic elements able to link  
40 resistance genes as gene cassettes. If integrons are located on plasmids or transposons they transfer  
41 linked resistance genes within or between bacterial species (Fluit and Schmitz, 2004). Linkage of  
42 resistance genes is considered to be involved in the transfer of MDR through co-selection by using  
43 individual antimicrobials (Fluit and Schmitz, 1999).

44 In both MDR *Salmonella* and MDR *E. coli* from broiler chickens plasmids containing class 1  
45 integron with gene cassettes *dfrA1-aadA1* is frequently found in the Netherlands (van Essen-  
46 Zandbergen et al., 2007; Vo et al., 2007), as well as in many other countries (Poppe et al., 2006;  
47 Riano et al., 2006). *In vitro* transfer of plasmids harbouring this integron from *E. coli* of avian origin and  
48 *Salmonella* to *E. coli* K12 has been described (van Essen-Zandbergen et al., 2007). Also *in vivo*  
49 transfer of plasmids in *Enterobacteriaceae* has been described in mouse intestine (Garcia-Quintanilla  
50 et al., 2008; Schjørring et al., 2008) and in turkey intestine (Poppe et al., 2005). However, information  
51 on *in vivo* transfer in young broiler chickens is lacking including the influence of the use of  
52 antimicrobials on this transfer. To study *in vivo* transfer of plasmid mediated class 1 integron  
53 containing gene cassettes *dfrA1-aadA1* and the influence of antibiotics an animal trial in young  
54 chickens was performed. Since doxycycline and trimethoprim/sulphamethoxazole are commonly used  
55 in broiler chickens, these antibiotics were used in the animal experiment.

56 The aim of this study was to determine *in vivo* transfer of a plasmid harbouring a class 1  
57 integron containing gene cassettes *dfrA1-aadA1* from *Salmonella* to *E. coli* in chickens with or without  
58 the influence of doxycycline or trimethoprim/sulphamethoxazole.

59

## 60 2. Materials and methods

61 2.1 Experimental design of the *in vivo* transmission studies

62 In the animal trial *S. enterica* spp. *enterica* serovar Typhimurium S115.62 containing a plasmid  
63 harbouring a class 1 integron with a 1550 bp size variable region harbouring gene cassettes *dfrA1*-  
64 *aadA1* was used as donor strain. This strain demonstrates resistance to amoxicillin, tetracycline,  
65 sulphamethoxazole and trimethoprim (van Essen-Zandbergen et al., 2007). In the animal trial *E. coli*  
66 K12 was used as recipient because of its ability to acquire a class 1 integron harbouring plasmid that  
67 contained gene cassettes *dfrA1*-*aadA1*. Moreover *E. coli* K12 can be distinguished from commensal *E.*  
68 *coli* due to its rifampicin resistance and because it is indol negative. Bacterial cultures were grown  
69 overnight at 37°C on Heart Infusion Agar (AcuMedia Manufactures, inc. Lansing, Michigan, USA) with  
70 5% sheep blood prior to colonisation of the chickens.

71 Before the animal experiment, as a pilot study, 5 four-days-old Specified Pathogen Free (SPF)  
72 White Leghorn American (WLA) chickens were inoculated orally with 10<sup>4</sup> CFU *E. coli* K12 and 7 days  
73 later with 10<sup>4</sup> CFU *S. Typhimurium* S115.62. The purpose of this pilot study was to demonstrate  
74 colonisation of young chickens with the laboratory adapted *E. coli* K12 and to study the effect of  
75 administration of a potentially pathogenic *S. Typhimurium*. On day 5, 8, 9 and 10 after hatching *E. coli*  
76 K12 was isolated from cloacal samples using dry cotton swabs. On day 15, 16, 17 and 21 after  
77 hatching *E. coli* K12, *S. Typhimurium* and transconjugants were isolated by inoculating cloacal  
78 samples on selective MacConkey agar plates. The selective MacConkey agar plates contained either  
79 no contain antibiotics, 50 mg/L of rifampicin (batch no. 085K1929), 256 mg/L of trimethoprim (batch  
80 no.064K1256) or a combination of 50 mg/L of rifampicin and 256 mg/L of trimethoprim. The antibiotics  
81 used for the selective plates were obtained from Sigma-Aldrich (Poole, Dorset, UK).

82 Each swab was suspended in 1 ml of buffered peptone water, which was considered to be the  
83 1:10 w/v suspension. The average weight of faecal samples on cloacal swabs taken from young  
84 chickens was 0.093 g ± 0.068 g. *E. coli*, *S. Typhimurium* and transconjugants were isolated from the  
85 undiluted, the 10<sup>-1</sup>, 10<sup>-2</sup> and 10<sup>-3</sup> diluted faecal suspension in a semi-quantitative manner. The viable  
86 count in the samples discriminated 5 different quantitative levels. Level 1 contained to 0-2 x 10<sup>2</sup>  
87 CFU/gram faeces; level 2 contained 2x10<sup>2</sup> - 6x10<sup>3</sup> CFU/gram faeces; level 3 contained 6x10<sup>3</sup> - 6x10<sup>4</sup>  
88 CFU/gram faeces; level 4 contained 6x10<sup>4</sup> - 6x10<sup>5</sup> CFU/gram faeces, and level 5 contained 6x10<sup>5</sup> -  
89 6x10<sup>6</sup> CFU/gram faeces.

90 To study the influence of antibiotics on *in vivo* transfer of the integron, 30 four-days-old SPF  
91 WLA chickens colonized with *E. coli* K12 were divided into 3 groups of 10 animals and placed in  
92 separate isolators on day 4 after hatching. On day 11 all chickens were inoculated orally with  $10^4$  CFU  
93 *S. Typhimurium* S115.62. From day 13 to day 17, 1 group of chickens was daily treated with 100 ppm  
94 doxycycline orally through the drinking water, 1 group was daily treated with 40 ppm trimethoprim and  
95 200 ppm sulphamethoxazole orally through the drinking water, and 1 group remained untreated  
96 (control group). On day 5, 6, 7, 10 through 25 after hatching *E. coli* K12, *S. Typhimurium* and  
97 transconjugants were isolated in a semi-quantitative manner from cloacal samples on selective  
98 MacConkey agar plates as described above. Doxycycline (Soludox 50%) and  
99 trimethoprim/sulphamethoxazole (Methoxazol-T) were obtained from Eurovet Animal Health BV,  
100 Bladel, the Netherlands.

101 The donor strain, the recipient strain and a selection of transconjugants were quantitatively  
102 tested for susceptibility to sulphamethoxazole, trimethoprim, tetracycline, amoxicillin, chloramphenicol,  
103 neomycin, cefotaxime, gentamicin and ceftazidime with the broth microdilution method according to  
104 the CLSI guidelines (NCCLS, 2004), *E. coli* ATCC 25922 was included for quality control purposes.

105 The antimicrobial resistance genes present in the donor strain, the recipient strain and in a  
106 selection of transconjugants were determined by the miniaturized microarray Indentibac AMR04 (VLA  
107 Weybridge, Surrey, UK ) as described previously (Batchelor et al., 2008).

108 The *in vitro* growth curves of the donor strain, the recipient strain and a selection of  
109 transconjugants were determined in Luria-Bertani medium (LB medium) by using the Bioscreen C (OY  
110 Growth Curves AB Ltd, Helsinki, Finland) following the instructions provided by the manufacturer. The  
111 donor strain, the recipient strain and the transconjugants were diluted  $10^{-1}$ - $10^{-8}$  in 200  $\mu$ l LB-medium,  
112 the Optical Density of all dilutions was measured every 5 min for 24 h.

113 The donor strain and the transconjugants were tested for the presence of integron class 1 and  
114 for the presence of the 1550 bp variable region harbouring the gene cassettes *dfraA1-aadA1* by PCR,  
115 as described previously (van Essen-Zandbergen et al., 2007).

116

117 *2.2 Plasmid characterization*

118 The plasmid content of donor strain and the transconjugants were analysed using PCR Based  
119 Replicon Typing (PBRT) with primer pairs targeting replicon regions, as described previously (Carattoli  
120 et al., 2005).

121 To determine the size of the plasmids, S1-PFGE (Barton et al., 1995) was used. Total DNA of  
122 the donor strain and transconjugants was incorporated into SeaKem Gold agarose (Cambrex Bio  
123 Science Rockland Inc., Rockland, ME, USA), and digested for 45 min at 37°C with 8 U S1-nuclease  
124 (Amersham Biosciences). PFGE was performed in a CHEF DRIII system on a 1% SeaKem Gold  
125 agarose gel using the following electrophoreses conditions: switch time 1-25 s, run time 17 h, voltage  
126 6 V, angle 120°. Low range and middle range PFGE size standards (New England BioLabs) were  
127 included as molecular weight standards. DNA was stained with ethidium bromide.

128

### 129 2.3 Pulse Field Gel Electrophoreses and hybridization with a DIG labelled *int11* probe

130 To confirm the identity of the isolated strains, *Xba*I -PFGE was performed on the original *S.*

131 Typhimurium donor strain and the original *E. coli* K12 recipient, as well as on the isolated *S.*

132 Typhimurium, *E. coli* and transconjugants, as described by PulseNet

133 ([http://www.cdc.gov/pulsenet/protocols/ecoli\\_salmonella\\_shigella\\_protocols.pdf](http://www.cdc.gov/pulsenet/protocols/ecoli_salmonella_shigella_protocols.pdf)).

134 DNA from the gel was transferred to a positively charged nylon membrane Hybond N+ (Amersham  
135 Biosciences) using a fast blotting system (Bio-Rad Laboratories) following the instructions provided by  
136 the manufacturer and cross-linked by baking at 80°C for 30 min. Hybridisation was performed  
137 overnight at 60°C in Church hybridisation buffer (Church and Gilbert, 1984) with a DIG-labelled *int11*  
138 PCR amplification product. Labelling the *int11* amplification product and detection was performed using  
139 the DIG-High Prime DNA Labelling and Detection Starter Kit I (Roche Applied Science) following the  
140 instructions provided by the manufacturer.

141

## 142 3. Results

### 143 3.1 *In vivo* transmission study

144 In the pilot study (data not shown) and in the *in vivo* transmission study (Figure 1a, b and c) *E.*

145 *coli* K12 and *S. Typhimurium* were able to colonize the chickens' intestine immediately after

146 inoculation and could be re-isolated in high rates of approximately  $6 \times 10^5$  -  $6 \times 10^6$  CFU/gram faeces.

147 The chickens showed no clinical signs of disease other than a slight and transient diarrhea after

148 inoculation with *S. Typhimurium*. During the experiment only the original *Salmonella* donor strain, the  
149 original *E. coli* K12 recipient strain and *E. coli* K12 transconjugants were isolated from the chicken  
150 intestines.

151 In the present study transconjugants were observed almost immediately after inoculation with  
152 *S. Typhimurium* in the treated as well as in the untreated chickens (Figure 1a, b and c). This  
153 demonstrates that a high efficiency of *in vivo* conjugation existed. Treatment of the chickens with  
154 antibiotics had neither a positive nor a negative effect on the transfer rates.

155 Within 3 days after introduction of *S. Typhimurium* to the chickens' intestine (14 days after  
156 hatching) the viable count of the isolated rifampicin and trimethoprim resistant *E. coli* was equal to the  
157 total viable count of isolated *E. coli*. In the untreated group the viable count was equal on day 15 after  
158 hatching, demonstrating that all recipients became transconjugants and that no other commensal *E.*  
159 *coli* were isolated.

160

### 161 3.2 Pulse Field Gel Electrophoreses and hybridization with a DIG labelled *intI1* probe

162 Figure 2 shows the *Xba*I-PFGE profiles from the original *E. coli* recipient, the original *S.*  
163 *Typhimurium* donor strain as well as the profiles from a selected number of *Salmonella* strains re-  
164 isolated from the chickens and a number of isolated transconjugants. The profiles of the *Salmonella*  
165 strains re-isolated from chickens were similar to the profile of the original *S. Typhimurium*,  
166 demonstrating that the original *S. Typhimurium* was able to colonize in the chickens' intestine. The  
167 profiles of all isolated transconjugants were almost similar to the profile of *E. coli* K12, except for an  
168 extra band of approximately 50 kb in the profile of the transconjugants. The *intI1*-DIG labelled probe  
169 hybridized with this 50 kb band of the transconjugants as well as with a 50 kb band of the *Salmonella*  
170 strains (results not shown) confirming the presence of a class 1 integron on a transferable element.

171

### 172 3.3 Characterization of the isolates

173 PCR amplification showed that all re-isolated *Salmonella* strains and all transconjugants  
174 contained a class 1 integron with a 1550 bp variable region harbouring gene-cassettes *dfrA1-aadA1*.

175 Similar to the *S. Typhimurium* donor strain, all isolated transconjugants demonstrated  
176 resistance to amoxicillin, tetracycline, sulphamethoxazole and trimethoprim. The miniaturized  
177 microarray showed the presence of the resistance genes *aadA1*, *dfrA1*, *intI1*, *sul1*, *sul2*, *tem1*, *tetA*,



178 *strA* and *strB* in the donor strain and in the transconjugants as well. All isolated transconjugants were  
179 indol negative similar to the *E. coli* K12 recipient.

180 Transconjugants and *E. coli* K12 showed identical growth abilities *in vitro*. Difference was  
181 observed between the growth curves of the *S. Typhimurium* donor strain and the transconjugants, *S.*  
182 *Typhimurium* reached the log-phase and the stationary-phase approximately 2 hours sooner than the  
183 transconjugants.

184 S1-PFGE and hybridization of the donor strain and transconjugants showed the presence of a  
185 130 kb sized plasmid (results not shown), which hybridized with the *int1*-DIG labelled probe. This  
186 plasmid was identified as IncFIB by PBRT.

187

#### 188 4. Discussion

189 *In vitro* transfer of a plasmid harbouring a class 1 integron with gene cassettes *dfrA1-aadA1*  
190 from *E. coli* and *Salmonella* to *E. coli* K12 has been described (van Essen-Zandbergen et al., 2007).  
191 The present study demonstrates the *in vivo* plasmid mediated transfer of this integron from an  
192 amoxicillin, streptomycin, tetracycline, sulphamethoxazole and trimethoprim resistant *Salmonella*  
193 *Typhimurium* S115.62 to *E. coli* K12. In addition to the resistance genes *aadA1* and *dfrA1* encoding for  
194 streptomycin and trimethoprim resistance located on the variable region of the integron and the  
195 resistance gene *sul1* encoding sulphamethoxazole resistance located the 3' conserved segment of the  
196 integron, resistance genes *strA*, *strB*, *sul2*, *tem1* and *tetA* encoding for streptomycin, sulphanomide,  
197 amoxicillin and tetracycline resistance transferred from the donor strain as well.

198 Transconjugants were observed almost immediately after inoculation with *S. Typhimurium* in  
199 treated as well as in untreated chickens. This demonstrates a high efficiency of *in vivo* conjugation,  
200 similar data were described previously (Garcia-Quintanilla et al., 2008; Poppe et al., 2005)

201 Because genes encoding for resistance to tetracycline, trimethoprim and sulphamethoxazole  
202 were co-transferred on the 130 kb IncFIB plasmid, the doxycycline and  
203 trimethoprim/sulphamethoxazole used in the experiment had no inhibitory effect on the *S.*  
204 *Typhimurium* donor strain nor on the transconjugants but may have affected the growth of the  
205 recipient *E. coli* K12 (figure 1). The recipient *E. coli* K12 could not be re-isolated from chickens after  
206 colonization of *S. Typhimurium* in the chickens' intestine, as described previously (Schjørring et al.,  
207 2008). This may be due to a reduction in viable count of the recipient *E. coli* K12 below the detection

208 limit of 200 CFU/g faeces, by the inhibitory or killing effect of the antibiotics administered or by the very  
209 efficient and fast *in vivo* conjugation to all recipient *E. coli* K12 strains. This was observed in the  
210 chickens treated with antibiotics as well as in the untreated chickens. In contrast, transconjugant *E.*  
211 *coli* strains were able to colonize the chickens intestine very efficiently, indicating that the  
212 transconjugant were better adapted to the chicken intestines compared to the original *E. coli* K12  
213 isolate. Because transconjugants and *E. coli* K12 showed identical growth abilities *in vitro* these data  
214 suggest that in addition to genes encoding for antibiotic resistance also genes facilitating colonization  
215 of bacteria in chickens were transferred from *S. Typhimurium* to *E. coli*, which was previously  
216 described to occur in mice (Rice et al., 2009). Maybe the transfer of these colonization genes  
217 contributed to the success of the transfer of plasmids. Since in this study, antibiotics had no effect on  
218 the transfer rates of the plasmids. The study of Schjørring et al. (Schjørring et al., 2008) showed that  
219 during treatment with antibiotics plasmids were transferred to indigenous *E. coli* and treatment with  
220 antibiotics had effect on the colonization rates of *Klebsiella pneumoniae*. But in this study animals with  
221 a normal bacterial flora were used, while the present study used SPF chickens instead. This may  
222 explain the different results observed according to the antibiotic use.

223 It can be concluded that *in vivo* transmission of a IncFIB plasmid harbouring a class 1 integron  
224 with gene cassettes *dfrA1-aadA1* from *S. Typhimurium* to *E. coli* occurred in high efficiency in young  
225 SPF chickens with or without selection pressure by antibiotics.

226

## 227 **Acknowledgements**

228 Positive controls for the PCR Based Replicon Typing of the plasmids were kindly provided by  
229 Alessandra Carattoli from the Istituto Superiore di Sanità, Rome, Italy.

230

## 231 **Conflict of interest statement**

232 None to declare

233

## 234 **Funding**

235 This study was part of project no. 802.190.5000, Characterization and transmission of  
236 resistance genes, funded by the Ministry of Agriculture, Nature and Food Quality.

237

238 **Ethics approval**

239 In accordance with the Act on Experimental Animals, use of experimental animals is granted  
240 to CVI by permit from the Dutch Government. Every experimental use is evaluated and scrutinised by  
241 an Ethical Review Committee (ERC). This Committee evaluates ethical issues of animal experiments,  
242 which are likely to cause pain or distress to experimental animals. Special attention is paid on  
243 Replacement, Reduction and Refinement. The animal experiments are carefully planned in terms of  
244 design and execution, thereby minimising the amount of distress for animals. The Dutch study plan  
245 describing the animal experiment with number 2006128c was approved by the ERC.

247 **References**

- 248 Barton, B.M., Harding, G.P., Zuccarelli, A.J., 1995, A general method for detecting and sizing large  
249 plasmids. *Anal Biochem* 226, 235-240.
- 250 Batchelor, M., Hopkins, K.L., Liebana, E., Slickers, P., Ehricht, R., Mafura, M., Aarestrup, F., Mevius,  
251 D., Clifton-Hadley, F.A., Woodward, M.J., Davies, R.H., Threlfall, E.J., Anjum, M.F., 2008,  
252 Development of a miniaturised microarray-based assay for the rapid identification of  
253 antimicrobial resistance genes in Gram-negative bacteria. *Int J Antimicrob Agents* 31, 440-451.
- 254 Box, A.T., Mevius, D.J., Schellen, P., Verhoef, J., Fluit, A.C., 2005, Integrons in *Escherichia coli* from  
255 food-producing animals in The Netherlands. *Microb Drug Resist* 11, 53-57.
- 256 Carattoli, A., Bertini, A., Villa, L., Falbo, V., Hopkins, K.L., Threlfall, E.J., 2005, Identification of  
257 plasmids by PCR-based replicon typing. *J Microbiol Methods* 63, 219-228.
- 258 Church, G.M., Gilbert, W., 1984, Genomic sequencing. *Proc Natl Acad Sci U S A* 81, 1991-1995.
- 259 Fluit, A.C., Schmitz, F.J., 1999, Class 1 integrons, gene cassettes, mobility, and epidemiology. *Eur J*  
260 *Clin Microbiol Infect Dis* 18, 761-770.
- 261 Fluit, A.C., Schmitz, F.J., 2004, Resistance integrons and super-integrons. *Clin Microbiol Infect* 10,  
262 272-288.
- 263 Garcia-Quintanilla, M., Ramos-Morales, F., Casadesus, J., 2008, Conjugal transfer of the *Salmonella*  
264 *enterica* virulence plasmid in the mouse intestine. *J Bacteriol* 190, 1922-1927.
- 265 Leverstein-van Hall, M.A., HE, M.B., AR, T.D., Paauw, A., Fluit, A.C., Verhoef, J., 2003, Multidrug  
266 resistance among *Enterobacteriaceae* is strongly associated with the presence of integrons  
267 and is independent of species or isolate origin. *J Infect Dis* 187, 251-259.

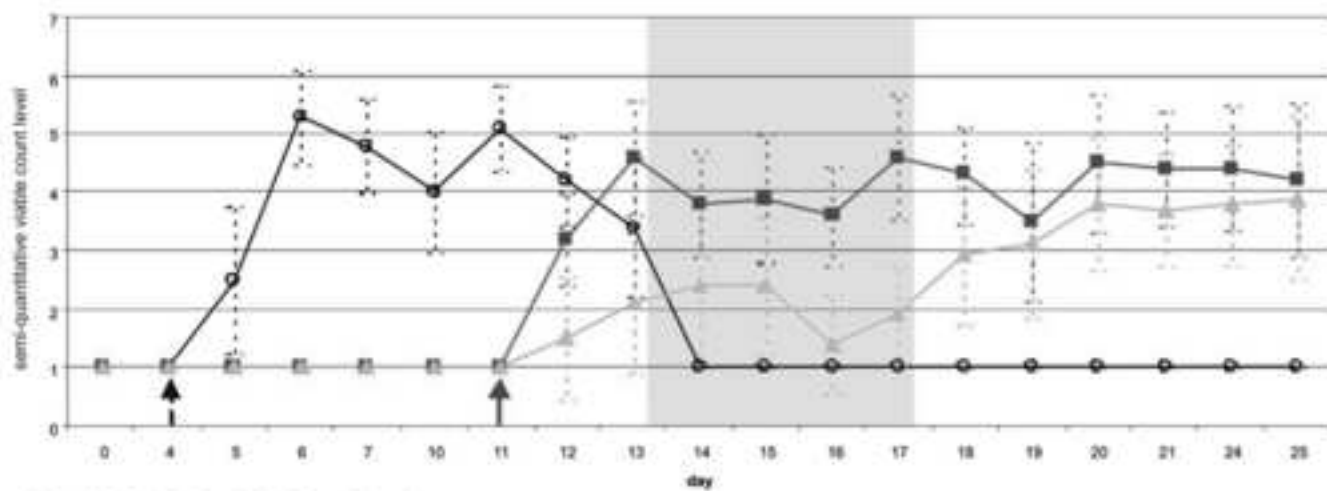
- 268 NCCLS, N.C.f.C.L.S. 2004. Performance Standards for antimicrobial susceptibility testing M31-A2.  
269 (Wayne. PA,USA).
- 270 Poppe, C., Martin, L., Muckle, A., Archambault, M., McEwen, S., Weir, E., 2006, Characterization of  
271 antimicrobial resistance of *Salmonella* Newport isolated from animals, the environment, and  
272 animal food products in Canada. *Can J Vet Res* 70, 105-114.
- 273 Poppe, C., Martin, L.C., Gyles, C.L., Reid-Smith, R., Boerlin, P., McEwen, S.A., Prescott, J.F.,  
274 Forward, K.R., 2005, Acquisition of resistance to extended-spectrum cephalosporins by  
275 *Salmonella enterica* subsp. *enterica* serovar Newport and *Escherichia coli* in the turkey poult  
276 intestinal tract. *Appl Environ Microbiol* 71, 1184-1192.
- 277 Riano, I., Moreno, M.A., Teshager, T., Saenz, Y., Dominguez, L., Torres, C., 2006, Detection and  
278 characterization of extended-spectrum beta-lactamases in *Salmonella enterica* strains of  
279 healthy food animals in Spain. *J Antimicrob Chemother* 58, 844-847.
- 280 Rice, L.B., Lakticova, V., Carias, L.L., Rudin, S., Hutton, R., Marshall, S.H., 2009, Transferable  
281 Capacity for Gastrointestinal Colonization in *Enterococcus faecium* in a Mouse Model. *J Infect*  
282 *Dis* 199, 342-349.
- 283 Schjørring, S., Struve, C., Krogfelt, K.A., 2008, Transfer of antimicrobial resistance plasmids from  
284 *Klebsiella pneumoniae* to *Escherichia coli* in the mouse intestine. *J Antimicrob Chemother* 62,  
285 1086-1093.
- 286 van Essen-Zandbergen, A., Smith, H., Veldman, K., Mevius, D., 2007, Occurrence and characteristics  
287 of class 1, 2 and 3 integrons in *Escherichia coli*, *Salmonella* and *Campylobacter* spp. in the  
288 Netherlands. *J Antimicrob Chemother* 59, 746-750.
- 289 Vo, A.T., van Duijkeren, E., Fluit, A.C., Gaastra, W., 2007, A novel *Salmonella* genomic island 1 and  
290 rare integron types in *Salmonella* Typhimurium isolates from horses in The Netherlands. *J*  
291 *Antimicrob Chemother* 59, 594-599.
- 292
- 293
- 294

295 Fig. 1. Dynamics of the mean viable count levels of *E. coli* K12 (○), *S. Typhimurium* S115.62 (■) and  
296 transconjugants (▲) in 10 broiler chickens treated ( ) orally with 100 ppm doxycycline (a); 40 ppm  
297 trimethoprim and 200 ppm sulphamethoxazole (b); untreated, control group (c). The broiler chickens  
298 were orally inoculated with *E. coli* K12 (▲) and with *S. Typhimurium* S115.62 (▲). Semi-quantitative  
299 viable count level 1 samples contained  $<2 \times 10^2$  CFU/gram faeces; level 2, samples contained  $2 \times 10^2 -$   
300  $6 \times 10^3$  CFU/gram faeces; level 3 samples contained  $6 \times 10^3 - 6 \times 10^4$  CFU/gram faeces; level 4 samples  
301 contained  $6 \times 10^4 - 6 \times 10^5$  CFU/gram faeces; and level 5 samples contained  $6 \times 10^5 - 6 \times 10^6$  CFU/gram  
302 faeces.  
303  
304

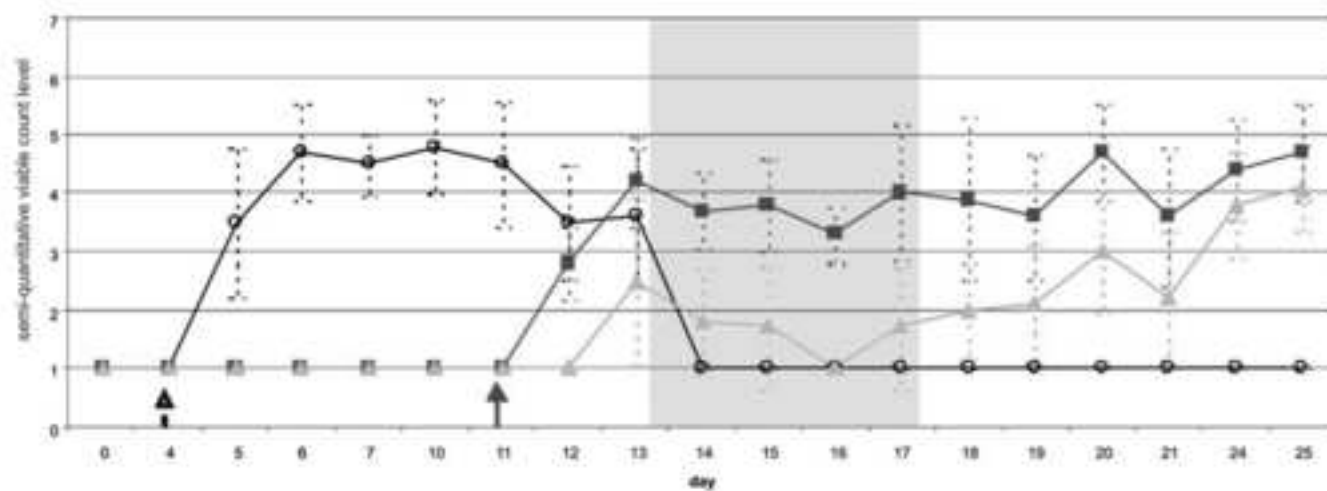
Accepted Manuscript

305 Fig. 2. Analysis of *E. coli*, *Salmonella* and *E. coli* transconjugants by *Xba*I-PFGE. Profiles obtained  
306 from: lane SB, *Salmonella* Braenderup (marker); lane 1, *E. coli* K12 from the inoculum; lane 2, *E. coli*  
307 isolated from chickens on day 6 after hatching; lane 3, transconjugant isolated from doxycycline  
308 treated group on day 20 after hatching; lane 4, transconjugant isolated from  
309 trimethoprim/sulphamethoxazole treated group on day 20 after hatching; lane 5, transconjugant  
310 isolated from the control group on day 24 after hatching; lane 6, *S. Typhimurium* S115.62 from the  
311 inoculum; lane 7, *Salmonella* isolated from the doxycycline treated group on day 20 after hatching;  
312 lane 8, *Salmonella* isolated from the trimethoprim/sulphamethoxazole treated group on day 20 after  
313 hatching; lane 9, *Salmonella* isolated from the control group on day 20 after hatching. The arrows  
314 show hybridization with the *int1*-probe.

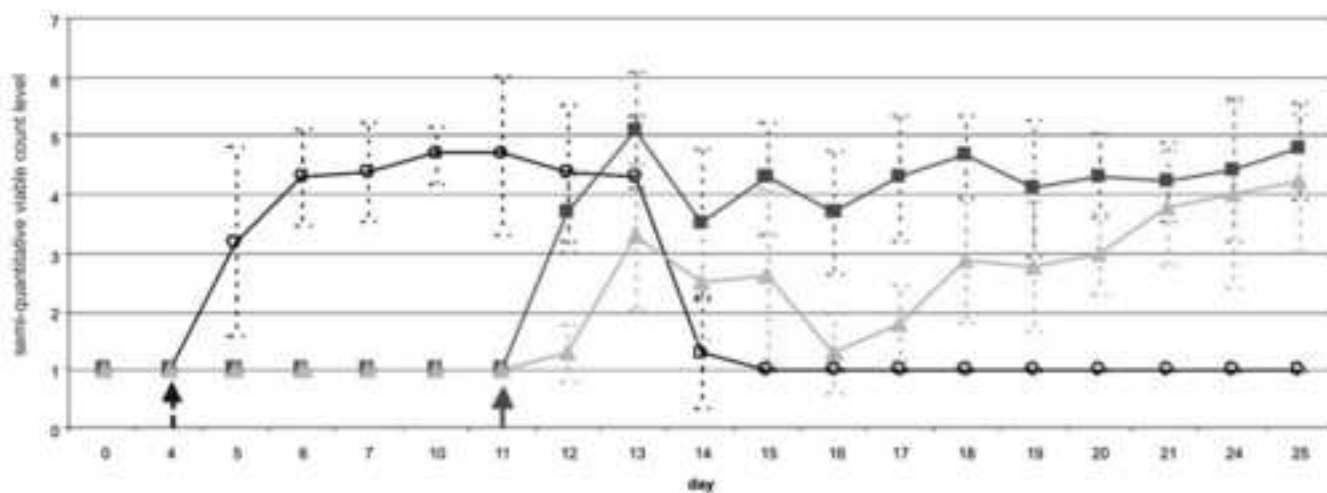
a. Treated with doxycycline



b. Treated with trimethoprim/sulphamethoxazole



c. Untreated, control group



trip

