Goedkeuring penvoerder / consortium	
De penvoerder heeft namens het consortium de jaarrapportage	V goedgekeurd □ niet goedgekeurd
Evt. opmerkingen over de jaarrapportage:	

Algemene gegevens	
PPS-nummer	AF-14210
Titel	1H4F- Reduction of ESBLs: evaluation of ESBL interventions
Topsector en innovatiethema	Theme 4.B. Sustainable livestock production, prevention of animal disease
Projectleider (onderzoek)	Daniela Ceccarelli
PPS-coördinator (namens private partij)	Dhr. Toon van Hoof, LTO Nederland
Contactpersoon overheid	Dr. Mark de Bode
Status (lopend of afgerond)	Lopend
Type onderzoek (F, T of V)	**F, T & V**
Werkelijke startdatum	1-1-2015 (we zijn eigenlijk begonnen op 1-6-2015)
Werkelijke einddatum	31-12-2018
Organisatie- / bestuursstuctuur	Het 1H4F consortium kent een 3-lagige organisatie- en governancestructuur: 1. Stuurgroep, 2. Adviesgroep, en 3. Projectgroepen. Zie voor meer informatie de oplegnotitie van het 1H4F consortium.
Begeleidingsstructuur (klankbordcie., etc.)	Zie boven
Korte omschrijving inhoud (max. 4 regels)	This project investigates scientifically based intervention strategies aimed at reducing colonization and spread of ESBL producing bacteria between animals and the different levels of the broiler production pyramid.

Planning en voortgang	
Loopt de PPS volgens planning?	From the start of the project AVINED budget has not been available for years 2015 and 2016 (125K), limiting our resources for financing staff at WBVR and UU. *in kind* contribution was provided by UU to cover salary costs. In 2016, additional budget (42.1K) was provided by the 1H4F funded project ESBL-Dynamics (P.L. Dik Mevius) and in 2017

	Avined participated to the budget of UU with 70K. During the annual stakeholders meeting (27/11/2017), Erik de Jong (Avined) updated the group on the current status of the AVV and clearly expressed the financial commitment of Avined to this project. New discussion and renegotiation of the budget with Avined will start after a decision on the AVV is taken by the Dutch Government (March 2018). The PhD student went on maternity leave as of December 7th ,2017 and is expected to return to work on April 2018.
Zijn er wijzigingen in het consortium/de projectpartners?	No
Is er sprake van vertraging en/of uitgestelde opleverdatum?	There is a slight delay compared to the original plan: 1. The PhD student (Mrs. Anita Dame-Korevaar) started in June instead of January 2015. 2. Because of the partial financing, animal experiments and testing has been reduced with limited choice for inoculation doses, routes and transmission.

Link naar samenvatting Kennis Online: https://www.wur.nl/nl/project/1H4F-Reduction-of-ESBLs-1.htm

Highlights

Intervention strategies that can prevent or reduce colonization and spread of extended spectrum beta-lactamase (ESBL) producing *E. coli* in the poultry production chain are here investigated. The target is specific on non-pathogenic ESBL producing *E. coli*. Other bacteria are not considered, although results might apply to these as well, considering the bacterial flora as a whole.

The PhD plan financed by this PPS-project is organized in the following tasks.

1. **Systematic Literature Review** (SLR). All available literature on the spread of ESBL producing bacteria within and between flocks in the poultry production pyramid was examined to list transmission routes for ESBL spread and related interventions. Out of 5651 screened articles, 86 were selected based on the presence of a defined or suggested ESBL transmission mechanism. The most important routes were defined as: (pseudo) vertical transmission between generations, transmission via hatcheries, horizontal (on farm) transmission, and horizontal between farm transmission (via the environment). A concept paper has been written and was submitted for publication to the journal Preventive Veterinary Medicine in October 2017 and is currently being evaluated by the Editor after receiving peer-review.
2. **Development of an animal model to test interventions**. To test the effect of interventions we have developed a reproducible animal model to define the lowest dose resulting in gut colonisation by ESBL producing *E. coli*. Five rounds of animal experiments (4 days each) were carried out to investigate the effect of different inoculation doses by two different ESBL producing *E. coli* strains (carrying diverse ESBL genes and plasmids) on gut colonization and excretion in young chicks. Both SPF and commercial chicks were used. Results showed a clear positive dose-response relationship between inoculation dose and time until excretion, which is an indication of colonization. Commercial chicks were colonized later (up to 60h) than SPF chicks (up to 24h), likely due to competition of pre-existing flora and inoculum strain for the same ecological niche. No differences were observed in colonization or excretion using different ESBL producing strains. Data have been analysed and writing of a scientific article is ongoing. Submission is expected in Spring 2018.
3. **Choice of intervention**. Based on the SLR, interventions to be applied in the animal model and, if effective, in the field have been discussed. Possible interventions can be subdivided with an aim to reduce exposure and contamination (e.g. compartmentalisation of the stable, egg decontamination), colonization (e.g. feed additive, competitive flora, acidification of feed) or excretion (e.g. competitive flora, phage therapy) of ESBL producing bacteria. In close dialogue with the industrial partner, prolonged application of competitive flora has been selected as the first intervention. As preventing colonization seems the most important step in reducing ESBL producing bacteria prevalence, this intervention has been investigated via the concept of competitive exclusion, as explained in Task 4.
4. **Testing of prolonged administration competitive flora in the animal model**. 3 rounds of animal experiments were carried out between April and November 2017 to determine the effect

on transmission and excretion of ESBL producing bacteria of prolonged (21 days) supply through drinking water of two different commercial competitive exclusion products (Aviguard or Poultrystar). As no differences were observed between different ESBL producing *E. coli* strains (see Task 2), one ESBL producing *E. coli* strain and two inoculation doses (10^1, 10^2 ESBL/ml) were used in all rounds. SPF and commercial chicks were used. Initial data analysis showed that prolonged administration of competitive flora does not have an effect if provided after inoculation of ESBL producing *E. coli* (1h after bacterial inoculation, round 1 with Aviguard), whereas it reduces time until colonization and excretion when provided in advance (4 days) before inoculum (round 2 with Aviguard). Administration of Poultrystar before ESBL inoculation (round 3) showed reduction on ESBL excretion and transmission rate, implying that competitive flora should be given as soon as possible, before the chicks come into contact with the ESBLs.

5. **Mathematical models**. The population level effect of the interventions will be simulated with two separate mathematical models, one describing within-flock transmission and one describing between-flock transmission. The latter model will be based on the poultry pyramid being a linked system with multiplications points and entry from the environment. This model will be a discrete-time stochastic model for which a flock is the epidemiological unit. This model is being developed in dialogue with the private partners and will be calibrated on existing information.

The within-flock transmission model will be used on the standard SIS model for which parameters can be estimated in the experiments. The experimental results can thus be extrapolated to larger populations. Read-outs from this model will be probability of colonization of a population and prevalence during the round. These can then be used as parameters for the in between-flock transmission model.

6. **The most promising interventions will be tested in the field.** During the annual stakeholders meeting (27/11/2017) it was agreed that the best intervention is a combination of methods where hygiene is essential. Using experimental pens to do controlled and repeatable experiments seems to be the approach to follow as field interventions would be too expensive, given the budget constraints. Either UU or Aviagen Spelderholt facilities will be used based on experimental set up. As clearly discussed, this will depend on budget renegotiation with Avined.

Maatschappelijke toepassing

The aim of the project is to find intervention strategies that can be applied in the poultry sector to reduce ESBL producing bacteria. The interventions can be aimed both at preventing poultry colonization as well as at reducing ESBL producing bacteria spread between animals or between different levels of the poultry production system (i.e. hatchery, fattening farms, etc.). Within the project, an animal model was developed to test the effect of interventions using different (natural) infectious routes. Successful interventions from the experiments will be tested under field conditions.

Knelpunten

The main bottleneck is the lack of and doubts about funding which keeps the progress of the project uncertain. Avined only contributed with a budget of 70K in 2017 instead of 62.5K/y in 2015, 2016 and 2017. Budget renegotiation with Avined in 2018 should be successful to partially compensate for the lacking budgets of 2015/2016.

Aantal opgeleverde producten in 2017					
Wetenschappe-lijke artikelen	Rapporten	Artikelen in vakbladen	Inleidingen/ workshops/ invited lectures	Aangevraag-de octrooien /first filings	Spin-offs
Voor meer informatie zie bijlage					

Verwacht u het komende jaar een octrooiaanvraag?	NEE

Bijlage: Titels van de producten of een link naar de producten op een openbare website

Peer Reviewed Publications

1. Dynamics of CMY-2 producing *E. coli* in a broiler parent flock. Dame-Korevaar A, Fischer EAJ, Stegeman A, Mevius D, van Essen-Zandbergen A, Velkers F, van der Goot J. *Vet Microbiol.* ***2017****;203:211-214.*
2. Transmission routes of ESBL/AmpC producing bacteria in the broiler production pyramid, a systematic literature review. Dame-Korevaar, A., Fischer, E.A.J., van der Goot, J., Stegeman, A., Mevius, D. *Submitted to Preventive Veterinary Medicine* (**October 2017**).
3. Dose-response of ESBL/pAmpC-*E. coli* colonization and excretion in young broiler chicks. A. Dame-Korevaar, E.A.J. Fischer, J. van der Goot, F. Velkers, K. Veldman, D. Ceccarelli, D. Mevius, A. Stegeman. *In preparation*

Conference Proceedings

2017

1. A. Dame-Korevaar, E.A.J. Fischer, J. van der Goot, F. Velkers, D. Ceccarelli, D.J. Mevius, A. Stegeman. Dose-response of ESBL/AmpC-E. coli colonization and excretion in young broiler chicks. 2017, MedVetNet 2017: One Health: Zoonosis Emerging Threats, Guildford (UK). *Oral presentation*
2. E.A.J. Fischer, D. Klinkenberg, A. Dame-Korevaar, D. Ceccarelli, J.A. Van der Goot, C. Dierikx, D. Mevius. Predicting Dynamics of Extended-Spectrum Beta Lactamase producing E.coli in broilers. 2017, Modelling in Animal Health – ModAH, Nantes (France). *Oral presentation*
3. A. Dame-Korevaar, E.A.J. Fischer, J. van der Goot, F. Velkers, D. Ceccarelli, A. Stegeman, D. Mevius. Dose-response of ESBL/AmpC-E. coli colonization and excretion in young chicks. 2017, SVEPM Conference & Annual General Meeting of the Society, Inverness (UK). *Poster presentation*

2016

1. Dame-Korevaar, M.A., Fischer, E.A.J., van Essen-Zandbergen, A., Veldman, K.T., Stegeman, J.A., Mevius, D.J., van der Goot, J.A., Sharp decrease of CMY-2 producing E. coli in a broiler parent stock flock. 2016, Annual meeting of Society for Veterinary Epidemiology and Preventive Medicine (SVEPM), Elsinore (Denmark). *Poster presentation*
2. A. Dame-Korevaar. Dynamics of sharp decrease or CMY-2 producing *E. coli* in a broiler parent stock flock. 2016, The Scientific Spring Meeting KNVM & NVMM, Arnhem(the Netherlands). *Oral presentation*
3. A. Dame-Korevaar, EAJ Fischer, A. Van Essen-Zandbergen, KT Veldman, JA Stegeman, DJ Mevius and JA Van Der Goot. Sharp decrease of CMY-2 producing *E. coli* in a broiler parent stock flock. 2016. 16th International Conference on Production Diseases in Farm Animals, Wageningen (the Netherlands). *Oral presentation.*