Banning antibiotics, reducing resistance, preventing and fighting infections

White Paper on research enabling an 'antibiotic-free' animal husbandry

Met Nederlandse samenvatting: Intensieve veehouderij zonder antibiotica

WAGENINGEN UR
For quality of life

Universiteit Utrecht

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Colophon

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In the course of compiling this White Paper a workshop was held on May 20th 2009 at the Central Veterinary Institute in Lelystad. The following persons participated in this meeting:

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List of abbrevations

AHS	Animal Health Service
ASG	Animal Sciences Group
BRSV	Bovine Respiratory Syncytial Virus
BVDV	Bovine Viral Diarrhoea Virus
EFSA	European Food Safety Authority
FIDIN	Fabrikanten en Importeurs van Diergeneesmiddelen In Nederland
KB	Kennisbasis
CVI	Central Veterinary Institute
DAS	Department of Animal Sciences
ESBL	Extended Spectrum β-lactamase
FAO	Food and Agriculture Organization
FVM	Faculty of Veterinary Medicine
KNMVD	Koninklijke Nederlandse Maatschappij voor Diergeneeskunde
LEI	Landbouweconomisch Instituut
LNV	Ministerie voor Landbouw, Natuur en Voedselkwaliteit
LR	Livestock Research
MRSA	Methicillin-resistant Staphylococcus aureus
OIE	Organisation Mondiale de la Santé Animale
PCV2	Porcine Circovirus Virus type 2
PI-3	Parainfluenza Virus type 3
PIA	Porcine Intestinal Adenomatosis
RIVM	Rijksinstituut voor Volksgezondheid en Milieu
PRRSV	Porcine Respiratory and Reproductive Syndrome Virus
VetCIS	Veterinair Centraal Informatie Systeem
VWA	Voedsel en Waren Autoriteit
WHO	World Health Organization

Contents

Nederlandse samenvatting

Intensieve veehouderij zonder antibiotica

Het groeiende antibioticagebruik in de intensieve veehouderij leidt tot een toename van antibioticaresistente kiemen zoals MRSA. Het vormt daarmee een serieuze bedreiging voor de gezondheid van mens en dier. Om dit probleem aan te pakken zullen strategische keuzes gemaakt moeten worden door politiek en bedrijfsleven. De partners van de Kennisketen Infectieziekten Dier (KID) hebben een White Paper opgesteld met daarin een aantal voorstellen voor nader onderzoek om vragen, die bij het maken van die keuzes aan de orde komen, te kunnen beantwoorden. Het White Paper geeft een overzicht van kennis die ontbreekt en draagt voorstellen aan om deze kennis te vergaren, over te dragen en toe te passen.

Aanpak

Ziektes vormen een constant risico voor de intensieve veehouderij. Antibiotica vormden een effectieve, betrouwbare en goedkope manier om ziekte te bestrijden of te voorkomen. Dit heeft tegelijkertijd geleid tot een overmatig gebruik van antibiotica en het heeft de ontwikkeling van preventieve bestrijdingsstrategieën en van alternatieven geremd. Tot voor kort waren er weinig redenen voor veehouders om het antibioticagebruik aan banden te leggen. Nu antibioticaresistentie als onvoorzien neveneffect zich steeds verder verspreidt, zoekt de sector naar alternatieven.

Gedeeltelijke oplossingen voor dit complexe probleem zijn echter niet afdoende. Om tot een uiteindelijke oplossing te komen moeten strategische keuzes gemaakt worden door politiek en bedrijfsleven. Multidisciplinair onderzoek, waarin technische, sociaaleconomische en culturele aspecten geïntegreerd worden, kan daarbij instrumenteel zijn.

Het primaire doel van het voorgestelde onderzoek is veehouders in staat te stellen infecties te voorkomen en de diergezondheid te controleren met een minimale hoeveelheid antibiotica. Dit minimaliseert het ontstaan en de verspreiding van antibioticaresistente bacteriën. Om dit te bereiken is een drastische omslag nodig in de ziektepreventie en –bestrijding. Het verminderen van antibioticagebruik in de veehouderij vraagt dan ook gedragsveranderingen van de betrokkenen. De KID heeft als ambitie door middel van onderzoek technische oplossingen aan te dragen die zulke gedragsveranderingen mogelijk maken. In dit White Paper wordt daartoe een aantal voorstellen geformuleerd.

MRSA

MRSA is de afkorting van Methicilline Resistente *Staphylococcus aureus*. Dit betekent dat deze bacterie ongevoelig is voor het antibioticum methicilline en ook voor de meeste andere antibiotica. Deze ongevoeligheid voor de meest gangbare antibiotica, maakt de bacterie moeilijk te bestrijden.
Er zijn heel veel verschillende typen (stammen) MRSA. Deze zijn in te delen in drie hoofdsoorten: ziekenhuis-MRSA, community-acquired MRSA en veegerelateerde MRSA. De ziekenhuis-MRSA is de 'klassieke variant' die kan worden opgelopen in ziekenhuizen en zorginstellingen. CA-MRSA staat voor Community Acquired-MRSA en wordt opgelopen buiten ziekenhuizen en zorginstellingen. Veegerelateerde MRSA is aangetroffen bij varkens, kalveren en pluimvee.
Het aantal jaarlijkse MRSA infecties stijgt snel. In 2008 waren 3038 gevallen van besmetting bekend, terwijl dit er in 2007 'slechts' 2619 waren. Zo'n 30 procent van deze infecties is aan vee gerelateerd.

Kennisketen Infectieziekten Dier

De Kennisketen Infectieziekten Dier (KID) is een samenwerkingsverband van de Animal Sciences Group van Wageningen UR, de faculteit Diergeneeskunde van de Universiteit Utrecht en de Gezondheidsdienst voor Dieren in Deventer. Afhankelijk van de projecten, worden andere partijen bij de samenwerking betrokken. Initiatiefnemer van dit samenwerkingsverband is het Ministerie van Landbouw, Natuur en Voedselkwaliteit (LNV). De kennisketen maakt optimale kennisontwikkeling mogelijk, waarbij de ontwikkelde kennis doorstroomt tussen de verschillende schakels van de keten naar de uiteindelijke gebruikers: dierenartsen en veehouders.

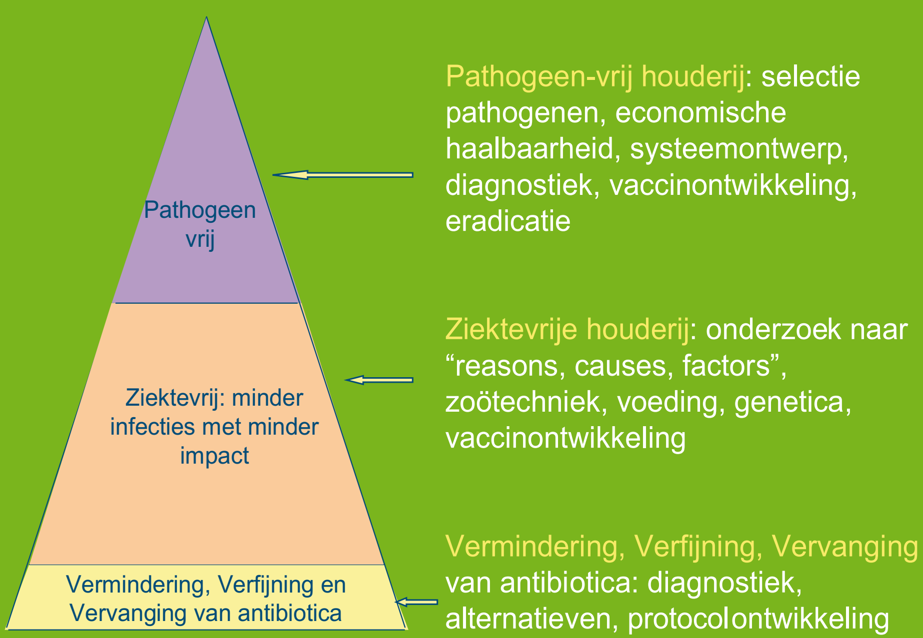
Werkpakketten

De KID heeft tien werkpakketen opgesteld die elk verwante onderzoeksvragen en technische benaderingen omvatten.

1. Onderzoek gericht op het identificeren van de bronnen van antibioticaresistentie. Voor deze bronnen worden preventie- en bestrijdingsstrategieën opgesteld.
2. Onderzoek gericht op een zo veel mogelijk pathogeenvrije inrichting van de veehouderij. Voorwaarde hiervoor is dat een duurzame pathogeenvrije status bereikt en onderhouden kan worden tegen redelijke kosten. De uitroeiing van pathogenen kan regionaal of per bedrijf of keten plaatsvinden.
3. Onderzoek gericht op het voorkómen van ziekte op veehouderijen met speciale aandacht voor huisvesting, verzorging en genetica. Uitgangspunt is dat de infectiedruk en de klinische en economische impact van infecties zoveel mogelijk verlaagd worden.
4. Onderzoek gericht op het optimaliseren van gezondheid door middel van voeding. Slechte voeding kan het immuunsysteem van vee negatief beïnvloeden en digestiestoornissen induceren. Daarom is goede voeding essentieel. Om gezondheideffecten van voeding te kunnen onderzoeken en voorspellen zullen modellen en parameters opgesteld worden.
5. Onderzoek gericht op een betere diagnostiek. Voor veehouders is het gebruik van antibiotica een kostenefficiënte manier om competitief te blijven. Maar antibiotica worden vaak ingezet zonder een specifieke diagnose van het pathogeen. Deze manier van

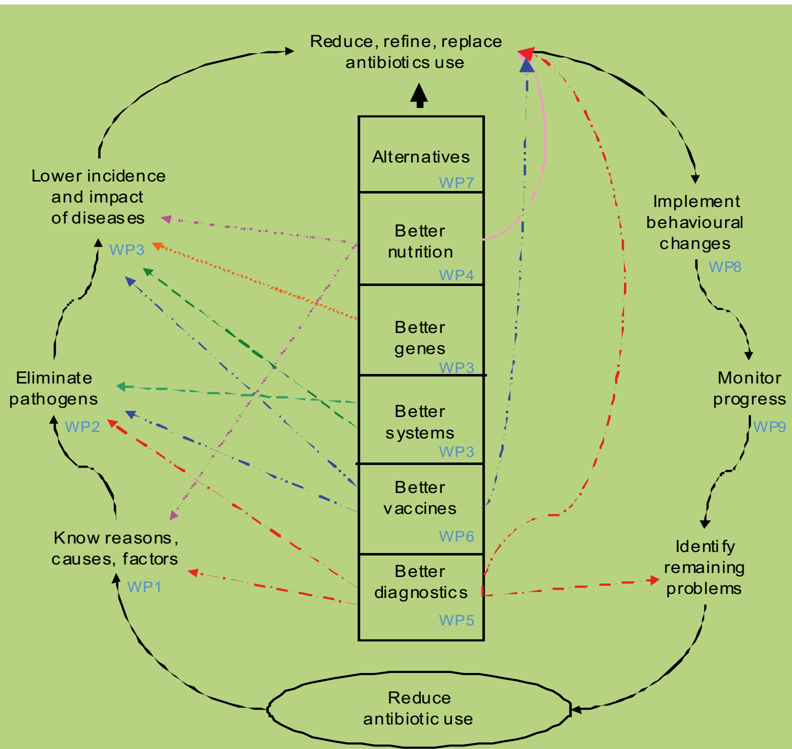
Om resistentie tegen antibiotica in de dierhouderij te verminderen kunnen we ons richten op het:

- *Verminderen van de noodzaak om antibiotica te gebruiken door het ontwerpen van een "pathogeen-vrije" houderij.*
- *Verminderen van de impact van die infecties die niet vermeden kunnen worden door het ontwerpen van een "ziekte-vrije" houderij, waarin minder infecties voorkomen en waar dieren de genetische en fysiologische mogelijkheden hebben om infecties te bestrijden met minimale klinische verschijnselen.*
- *Verminderen, verfijnen en vervangen van het gebruik van antibiotica om het risico op de ontwikkeling van antibacteriële resistentie te minimaliseren.*



antibioticagebruik kan flink teruggedrongen worden door diagnostische tests te verbeteren, te vergemakkelijken en goed gebruik ervan te stimuleren.

6. Onderzoek gericht op de ontwikkeling van vaccins voor een aantal specifieke veel voorkomende ziekten. Er zijn al veel goede, effectieve vaccins beschikbaar. Maar er zijn ook veel ziektes waar geen of geen effectief vaccin voor bestaat. Het ontwikkelen van vaccins tegen deze ziektes kan een flinke slag betekenen in het terugdringen van antibioticagebruik.
7. Onderzoek gericht op de ontwikkeling van alternatieven voor antibiotica. Om de verdere verspreiding van resistentie tegen te gaan, zijn alternatieve antibacteriële medicijnen die anders werken dan antibiotica een optie. Voorwaarde is dat het alternatief niet ook het risico van resistentie met zich meebrengt en dat het geen andere negatieve bijeffecten heeft voor mens en milieu. Opties zijn bijvoorbeeld bacteriofagen, de natuurlijke virussen van bacteriën en antibacteriële peptiden.
8. Onderzoek gericht op het induceren van gedragsveranderingen. Diverse technologische ontwikkelingen zullen kunnen bijdragen aan het verminderen van het antibioticagebruik. Het kan echter heel moeilijk zijn het gedrag rond antibioticagebruik te veranderen. Daarom moeten ook het diermanagement, de houding ten opzichte van ziektepreventie en medicijngebruik, en wetten en regels aangepast worden. Het is hierbij van belang de barrières te kennen die gedragsveranderingen in de weg staan.
9. Onderzoek gericht op het optimaliseren van de registratie van het antibioticumgebruik en het monitoren van resistentie. Registratie van het gebruik van antibiotica geeft een goede basis voor een interventiebeleid. Registratie gebeurt nu nog veelal op vrijwillige basis. De KID wil het registratieproces van antibioticumgebruik en de resistentiemonitoring verder ontwikkelen en optimaliseren.
10. Onderzoek gericht op een goede communicatie tussen onderzoek en praktijk. Om te zorgen dat wetenschappers in de verschillende werkpakketten hun resultaten goed op elkaar afstemmen, moet de communicatie soepel verlopen. Ook moeten de resultaten van het onderzoek snel naar de praktijk vertaald worden. De wetgeving voor antibioticagebruik wordt mogelijk gebaseerd op de EU-wetgeving. Daarom zoekt de KID ook samenwerking op Europees niveau, zowel met onderzoekers als beleidsmakers.



De relatie tussen werkpakketten en onderzoeksdoelen

Fasering

De KID is bereid om afhankelijk van de strategische keuzes die politiek en bedrijfsleven de komende periode zullen maken een samenhangend onderzoeksprogramma voor te leggen met behulp waarvan openstaande kennisvragen beantwoord kunnen worden. Het spreekt voor zich dat de termijn waarop vragen beantwoord kunnen worden zal afhangen van de gestelde vraag. Op korte termijn (1-3 jaar) kunnen de redenen van antibioticagebruik in kaart gebracht zijn en kan bekend zijn hoe het management van veehouderijen zodanig geoptimaliseerd kan worden dat sprake is van een minimale infectiedruk en gezondere dieren. Op middellange termijn (3 - 5 jaar) kan inzicht verkregen worden in het systeemontwerp voor een pathogeen- en ziektevrije dierhouderij, en kunnen snelle en goedkope diagnostica ontwikkeld worden zodat het antibioticagebruik beperkt kan worden. Daarbij kunnen inzichten verkregen worden om gewenste veranderingen daadwerkelijk te kunnen implementeren. Op wat langere termijn (> 5 jaar) kan inzicht verkregen worden in meer basale mechanismen van ziekteresistentie, nieuwe methoden van immuniteitsinductie, en zijn daadwerkelijk betere vaccinaties ontwikkeld.

Achtergrond

Nederland gebruikt relatief weinig antibiotica in de humane medische zorg om resistentie zoveel mogelijk te beheersen. Het therapeutisch veterinair antibioticagebruik is in Nederland echter veel hoger en ook hoger dan in andere landen. Resistente bacteriën ontstaan en verspreiden zich vooral op plaatsen waar veel antibiotica worden gebruikt, waar veel dieren dicht bij elkaar zitten en waar dieren veel worden verplaatst. Deze drie kenmerken tekenen de intensieve veehouderij.

Antibiotica in de Nederlandse veehouderij

Tussen 1999 en 2007 is het gebruik van antibiotica in de veehouderij toegenomen met 83%, tot 590.000 kg. In 2008 is er een daling opgetreden naar 520.000 kg. Het merendeel van de antibiotica komt terecht bij varkens, kalveren en vleeskuikens. Varkens krijgen de meeste antibiotica via het voer en drinkwater toegediend, kalveren via de melk, kippen via het drinkwater. Het gebruik per veehouder loopt sterk uiteen. Een deel van de veehouders geeft zijn vee op regelmatige basis antibiotica als ziektepreventie. Anderen passen antibiotica alleen toe als ziekte of infectie is vastgesteld.

In 2006 was dertig procent van de resistente bacteriën in pluimvee resistent tegen minimaal zes soorten antibiotica. Op 68% van de varkens- en 88% van de kalverhouderijen is de veegerelateerde MRSA-bacterie gevonden.

Er komen steeds meer aanwijzingen dat er overdracht van resistente bacteriën plaatsvindt van dieren naar de mens. Omdat in de veehouderij en de humane gezondheidszorg grotendeels dezelfde antibiotica worden gebruikt, betekent dit dat resistentie die in de veehouderij ontstaat ook de humane gezondheid kan bedreigen. De antibioticaresistentie in de humane medische zorg in Nederland is laag. Hierdoor heeft resistentie in de veehouderij een relatief grote impact.

De kans is groot dat resistente bacteriën zich verder zullen verspreiden tussen dieren en naar mensen. Dit zal ernstige gevolgen hebben bij de behandeling van infecties bij zowel mensen als dieren. Het in toom houden van resistente infecties in de publieke gezondheidszorg zal bovendien hoge kosten met zich meebrengen.

Ministerie van LNV

Resistentie bij dieren is een gevoelig onderwerp, omdat naast risico's voor dier- en volksgezondheid ook economische belangen een rol spelen. Het is voor de veehouder veelal goedkoper preventief antibiotica toe te dienen, dan structurele maatregelen te nemen om problemen te voorkomen. Om in de toekomst antibiotica te kunnen blijven gebruiken, zijn naast gericht onderzoek andere maatregelen noodzakelijk.

Het ministerie van LNV is al jaren voorstander van selectief en restrictief gebruik van antibiotica in de intensieve dierhouderij. Met dat doel heeft het ministerie van LNV in samenspraak met het ministerie van VWS de afgelopen jaren diverse acties in gang gezet. Recentelijk (in 2008) is de Taskforce Antibioticumresistentie Dierhouderij ingesteld. Dit heeft geleid tot een convenant om het antibioticagebruik te gaan terugdringen. Het werd in december 2008 ondertekend door de brancheorganisaties van de dierenartsen, de veehouders, de diergeneesmiddelenfabrikanten, de voerleveranciers en de vleesverwerkers. Een belangrijk element in dit convenant: een registratiesysteem voor diergeneesmiddelen. Hiermee ontstaat helderheid over het gebruik en kunnen veehouders en dierenartsen nagaan of ze meer antibiotica gebruiken dan hun collega's. Ook moeten de dierenartsen en veehouders samen een bedrijfsgezondheidsplan opstellen, om de ziektedruk op de bedrijven terug te dringen. Door beide maatregelen moet het bewustzijn over verantwoord antibioticagebruik toenemen. Minister Verburg van LNV heeft een commissie ingesteld die het convenant bewaakt.

De komende periode zal de politiek in samenspraak met het bedrijfsleven komen tot strategische keuzes voor de toekomst. Dit White Paper omvat een aantal voorstellen voor nader onderzoek om vragen die bij het maken van keuzes aan de orde komen te kunnen beantwoorden en om daadwerkelijke oplossingen aan te kunnen dragen. Het einddoel is daarbij helder: Op weg naar een dierhouderij die systematisch pathogenen zo "managet" dat antibioticagebruik minimaal zal zijn.

Consortium

De KID brengt alle partners, kennis en expertise samen die nodig is om het programma succesvol te kunnen uitvoeren. De partners hebben allemaal hun eigen niche in het onderzoeksveld en vullen elkaar uitstekend aan. Ook de infrastructuur die nodig is om kennis van de onderzoekswereld op een begrijpelijke manier naar het werkveld over te brengen is al nadrukkelijk aanwezig. De KID is daarmee de aangewezen partij voor onderzoek om het antibioticagebruik in de veehouderij sterk te verminderen en zo de kans op antibioticaresistente bacteriën in zowel de veterinaire als humane gezondheidszorg fors te verlagen.

Summary

Resistance of bacteria to antibiotics in animal husbandry is increasing and a point of growing concern. The large use of antibiotics in agriculture undoubtedly leads to the development of antibiotic resistance. This has resulted in a growing public concern on the rise of antibiotic resistance, and in particular on the transmission of resistant bacteria and resistance markers from animals to humans.

Large antibiotic use in animal husbandry and antibiotic resistance threatens the health and well being of man and animal through a diminished effectiveness of antibiotic treatments. It causes high costs of – unnecessary or ineffective – antibiotic treatments of animals, and it impairs the image and legitimacy of the intensive livestock sector resulting in a further decline of its societal support and the consumer's demand for its products. Therefore, politicians and industry will have to make forward looking choices.

In this White Paper we present work packages for research lines aimed at eliminating the systematic use of antibiotics in the animal production sector and therewith the emergence of, and selection for, antibiotic resistance. We consider it **urgent** that the animal husbandry will start producing **antibiotic-free** wherever and as much as possible. Such a development requires large changes in day-to-day practices, attitudes, and behaviour of all participating stakeholders in animal husbandry. Changes may be enabled by new technical solutions and a design of animal husbandry aimed at optimal disease prevention. It is an illusion that a simple solution will suffice to reduce antibiotic use in animal husbandry. Integrated, multidisciplinary and comprehensive approaches will be absolutely required to make progress. A "search-and-destroy" policy may further be necessary to combat remaining resistant bacteria after the use of antibacterials as selective force has been diminished.

This White Paper aims to identify knowledge gaps, and formulates research lines that may provide technical solutions towards antibiotic-free production. In addition it aims to formulate policy research aimed at implementing wanted changes. Part of the research lines may contribute to a reduction in antibiotic use on the short term. For a further reduction in antibiotic use, more strategic and long term research efforts will be needed.

It is the ambition of this White Paper to enable:

- To act routinely and to control infectious diseases without the use of antibiotics.
- To reduce the emergence of antibiotic resistance in animal husbandry.
- To reduce the transfer of resistance among animals and from animals to man.
- To use antibiotics (if necessary in case of accidental infections) in a prudent manner to minimize the emergence and prevalence of resistant bacteria.
- To combat resistant bacteria in animals when necessary.
- To prevent therapy failure and to ensure the effectiveness of necessary antibiotic treatments in animals and man.
- To increase the quality of veterinary preventive medicine.
- To improve the quality of animal husbandry.
- To improve the reputation and societal acceptance of animal husbandry.

For preventing the emergence of antibiotic resistance we consider it important to work on the following research goals.

A. Reduction of the need to use antibiotics in livestock production by designing a **"pathogen-free"** husbandry,

B. To diminish the impact of those infections that can not be avoided, by designing a **"disease-free"** animal husbandry, where fewer infections occur, and where animals

have the genetic, physiological and behavioural possibilities to combat infections with minimal clinical signs and economic losses should infection nonetheless occur.

C. **Reducing, refining, replacing** methods of application of antibiotics to minimize the risk of resistance in bacteria.
D. **Implementing changes** resulting in diminished use of antibiotics.
E. Development of methods to improve the **monitoring** of resistance.

For that purpose we propose the following research lines or work packages

1. Research aimed at improving our understanding of the factors that determine antibiotic use and the conditions for which large amounts of antibiotics are used, so that alternative ways of prevention and intervention can be designed. Major efforts will be directed at further understanding conditions such as the respiratory disease complex in fattening pigs and ill-defined digestive tract disorders in poultry and meat calves. This will define current "best practices" for a sparse use of antibiotics.

2. A subsequent line of research is directed at allowing farmers to work "pathogen-free" wherever that is feasible. Therefore a multidisciplinary systems design approach is needed.

3. For those infections that can not be avoided, research will be directed at preventive measures diminishing the incidence and clinical and economic impact of infections. This will be achieved by "disease-free" design of animal husbandry systems, taking the genetic make-up of animals into account.

4. Health-promoting nutrition, i.e. nutrition based on knowledge of feed components and feed additives that optimally help the digestive tract to function normally, to resist disturbances, to meet productivity requirements, and to resist infections.

5. Better diagnostics. To allow that antibiotics are used in a better prescribed manner for better defined conditions, allowing the emergence of fewer resistant bacteria, we will undertake efforts to improve the quality and speed of detection, and molecular characterization of causative bacteria, so that such diagnostic tools can be widely applied.

6. Major contributions to reduction in antibiotic use may also be expected from the development of vaccines against diseases that are responsible for large antibiotic consumption, including –amongst others- the multicausal respiratory disease complexes in swine, calves, and poultry, S. suis infections in pigs, and post weaning diarrhoea/ oedema disease. Together such approaches may both enhance resistance to disease and lower the exposure of animals to pathogens.

7. Although the focus of this White Paper is on disease prevention rather than treatment, we propose to examine alternatives for the use of antibiotics. In particular we will examine alternatives that do not compete with, or do not have disadvantages for their use in the public health sector (such as the emergence of resistance, or availability of last resource), and thus are in particular suited for use in animals.

8. In addition, we propose to examine governance and behavioural aspects, including cultural, educational, organizational and economic hurdles, directed on enabling and implementing a scarce and prudent use of antibiotics. Therefore research will be directed on methods of raising awareness and knowledge among farmers, veterinarians, and technical consultants, methods of supporting farmers to reach progress in diminishing the use of antibiotics, methods of benchmarking, and governance and behavioural aspects.

9. Research aimed at optimizing surveillance methods for quantification of antibiotic usage and resistance in populations. This will provide insight in the effect of measures, and also provide insight into the necessity and strategies needed to combat resistant bacteria that continue to circulate despite the restricted use of antibiotics. Monitoring at farm level will asses the effectiveness of specific intervention strategies.

10. Communication of scientific results. The research efforts aimed at reducing antibiotic use in animal husbandry are complex, manifold and multidisciplinary. It is therefore of utmost importance to specifically address communication and scientific exchange, and the distribution of findings to relevant stake holders such as veterinarians, farmers, and policy makers.

Because we may expect that regulations in the field of antibiotic use are based on EU laws, we consider it important to seek and promote collaboration at the European level, both with researchers and policy makers.

We feel that it is an urgent matter that antibiotic use in the animal production sector is reduced, and that research should – as quickly as possible- enable stakeholders to take their responsibility. In short, this White Paper aims to contribute to a **sustainable disease prevention** for a sustainable animal husbandry by reducing antibiotic use and antibiotic resistance.

Introduction:

The problem

Antibiotics have had tremendous success in treating and reducing bacterial diseases in man and animals, and thus have contributed significantly to their health and well being. Developed from the 1930's onwards, they are now indispensable in public and animal health care. However, as a result of selection, bacteria have acquired resistance to antibiotics through mutation and genetic transfer, which now seriously threatens their efficacy. Indeed, for every new antibiotic class developed, resistant bacteria emerged within a few years after its introduction (Harbottle et al 2006).

Resistance of bacteria against antibiotics is encoded by complex genetic determinants which are transmitted between bacteria. In addition to resistance to a single antibiotic, more bacteria become multi-drug resistant.

Selection for resistance takes place anywhere an antibiotic is present, especially where antibiotics are present with high densities of various microorganisms. The use of antibiotics in agriculture, antimicrobial products, and release into the environment all play roles in the development of antibiotic resistance (ASM 2009, FAO/OIE/WHO, 2003). It is generally accepted that a restricted use of antibiotics, together with a search-and-destroy policy of resistant bacteria when necessary, helps in reducing the prevalence of resistant bacteria.

Since 1 January 2006 the use of antibacterial growth promoters[1] in animal feeds for pigs and poultry is forbidden in the EU, but this use is still widely practiced throughout the world. The use of these agents appeared to support health in these species leading to an increase in performance of 2-4%. Nonetheless, in The Netherlands the use of antibiotics as antibacterial growth promoters was largely replaced by an increased therapeutic or preventive use of antibiotics at veterinary prescription (FIDIN 2008, MARAN 2007).

The therapeutic veterinary antibiotic use in The Netherlands has increased in the period 1999-2007 by 83 %. In 2007 the use of antibiotics in animal husbandry increased with 9 % compared with the previous year. Mostly used are the tetracyclines (60%), trimethoprim/sulphonamids and macrolides. The increased used of doxycycline, trimethoprim/sulphonamids and tylosin represented 90 % of the increased use in 2007. In 2007 590.000 kg antibiotics have been used in animal husbandry, 90 % of which have been used for oral “herd or batch treatments” (FIDIN 2008, Mevius 2008). In 2009 this declined to 520.000 kg. In particular swine, calves and broiler chickens receive the majority of the antibiotic treatments in the Netherlands. There are serious indications that the majority of the treated animals is not ”sick” – at least not clinically visible-, and serious concerns exist whether all of this use is meaningful or effective. In addition to therapeutic use, much of this antibiotic use is thus preventive use in animals at risk of infection, use aimed at obtaining growth gain, or use “in the sake of certainty”.

Overall resistance levels, both of mono-drug and multi-drug resistance, in isolates from animals are rising. Resistance is increasing both among food-borne pathogens and the commensal flora, which may act as a reservoir of resistance genes for the human reservoir.

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[1] Antibacterial growth promoters are antimicrobial substances applied in sub therapeutic doses in animal feed. They most of the time are member of the same pharmacological groups as therapeutic antibiotics.

A few examples from the MARAN-2007 report: Resistance levels of food-borne commensal *E. coli* are highest in broilers and raw poultry meat products, followed by veal calves, slaughter pigs and dairy cattle. In particular mulitresistant *E. coli* poses a threat for human health because of the widespread occurrence of *E. coli* in the gut. In 2006 30% of the resistant bacteria isolated from poultry were multi-drug resistant to more than 6 classes of antibiotics. In broilers and raw poultry meat products resistance of *E. coli* to cefotaxime, which is indicative of ESBLs, shows a rising trend and is now almost 17 %. In *Campylobacter jejuni*, a known zoonotic food-borne pathogen, resistance levels for ciprofloxacin and nalidixic acid are increasing (for up to > 60 %), in particular among isolates from broilers. Multidrug resistance in *Enterococcus* species is very common (i.e. up to 70 %) among isolates from veal calves, pigs, and broilers, but not from dairy cows (MARAN-2007).

The issue of antibiotic resistance of bacteria is clearly a major issue in public health where infections are increasingly difficult to treat due to this problem. There is a growing public concern on the rise of antibiotic resistance, and in particular on the transmission of resistant bacteria and/or resistance markers from animals to humans. Since the antibiotics used in man and animals belong to the same pharmaceutical groups, there is a clear need for a prudent use of antibiotics in animal husbandry.

The contribution of animal husbandry to the increasing antibiotic resistance among humans is difficult to quantify, but considerable for specific resistant organisms of concern (e.g. methicillin-resistant *Staphylococcus aureus* [MRSA]). Transmission of resistance from animals to man has indeed clearly been demonstrated for some bacteria, including fluoroquinolone-resistant *Campylobacter* and MRSA (Endtz et al 1991, Voss et al 2005), and may also be true for cephalosporin-resistant E. coli carrying Extended Spectrum β-lactamase (ESBL) resistance markers.

MRSAs are resistant to all β-lactam antibiotics and are often co-resistant to other antimicrobials. Recently a certain MRSA strain (ST398) has spread to a high prevalence among intensive animal production systems all over the world, and this strain is able to infect humans (EMEA 2009). Indeed MRSAs are now widely spread among swine and calf herds, and their owners and family members. On 88 % of the Dutch calf herds MRSA was detected in one or more calves or in stable dust. One in every three persons working in this industry carries MRSA, and livestock-associated persons must therefore undergo special precautions when they visit hospitals (http://www.rivm.nl/cib/infectieziekten-A-Z/infectieziekten/MRSA(methicilline-resistente-staphylococcus-aureus)/FAQ_MRSA.jsp#index_10).

Resistance to cephalosporins in human isolates is increasing at an alarming rate. The rise in cephalosporin resistance among human enteric pathogens is due to an ESBL type that also occurs in 16 % of the poultry isolates (MARAN-2007). An aggravating aspect is that this type of resistance is located on mobile genetic elements, which are easily transferable within and between bacterial species. A contribution from the animal reservoir to the increase in humans is currently investigated.

Altogether, the contribution of animal husbandry to the increasing antibiotic resistance among the Dutch population is reason for concern, because an animal reservoir is created where resistant organisms of concern can survive and disseminate.

Not only human health is at stake. It is highly likely that infections of animals become more difficult and less effective to treat. However, effectiveness of antibiotic therapy is not routinely monitored in animals. Nonetheless, the Animal Health Service (2009) reports significant rises in the percentage of resistant porcine pathogens, such as *Actinobacillus pleuropneumoniae, Bordetella bronchiseptica, Haemophilus parasuis, Pasteurella multocida, Streptococcus suis,* and *Staphylococcus hyicus*. For example, the percentage of flumequin-resistant *B. bronchiseptica* has increased from 6 % in 2005 to 32 % in 2008.

Urgency

The contribution of animal husbandry to the increasing antibiotic resistance among the Dutch population is reason for great concern. Although the major determinant for antibiotic resistance in human health care is still antibiotic usage in humans, a large animal reservoir where resistance determinants of concern are selected and widely disseminated may contribute to antibiotic resistance of human pathogens. Nonetheless the last decade the therapeutic veterinary antibiotic use has increased considerably, in particular in the intensive livestock production sector. Not only human health is threatened, but infections in animals will become more difficult and less effective to treat. Due to these developments the image and legitimacy of the intensive livestock sector can be seriously impaired and can result in further decline of its societal support and the consumer's demand for its products. Besides, the role of veterinary medicine in the prevention and control of diseases is under discussion. In designing the animal production sector of the future, it is therefore of utmost importance to handle pathogens in a more prudent way.

In our opinion it is of utmost important to act and to try banning the systematic use of antibiotics in the animal production sector with great urgency. Failure to do so will likely result in:

- Further transmission of resistant bacteria and resistance markers (in addition to MRSA) among animals and to humans. This may result in further societal isolation of carriers of resistant bacteria originating from the agricultural sector, very expensive measures to control resistant infections in the public health sector, and diminished effectiveness of antibiotic treatments of humans (in first instance, but not exclusively, of farmers themselves) resulting in a severe course of infection.
- Diminished effectiveness of antibiotic treatments of animals.
- Continued cover-up of failures in husbandry and disease prevention, and thus in a diminished urgency to enhance quality and establish a way of producing that is not systematically dependent on the use of antibiotics.
- Failure to save the costs of – unnecessary or ineffective – antibiotic treatments of animals.
- The image and legitimacy of the intensive livestock sector can be seriously impaired and can result in a further decline of its societal support and the consumer's demand for its products.
- Insufficient support to sustainability, both of the animal production sector and of veterinary medicine responsible for disease prevention and control.

Purpose and ambition

In this White Paper we make an inventory of knowledge gaps and present proposals to fill these knowledge gaps with research lines aimed at reducing systematic antibiotic use and antibiotic resistance in animal husbandry. It is thus our ambition to formulate research proposals that provide the animal husbandry sectors with the means and tools that enable:

- To act routinely (and control infectious diseases) without the use of antibiotics. Due to the possibilities of multi-drug or co-resistance the reduction of antibiotic use cannot be limited to a certain class of antibiotics, but should be directed on (the routine) use of antimicrobials in general. Designing a pathogen-free and disease-free animal husbandry are therefore key activities.
- To reduce the emergence of and selection for antibiotic resistance in the bacterial flora of animals, as well as the transfer of resistant bacteria and resistance determinants among animals, and from animals to man.
- To use antibiotics (if necessary in case of accidental infections) in a prudent manner to minimize the emergence and prevalence of resistant bacteria.
- To combat resistant bacteria in animals when necessary.
- To prevent therapy failure and to ensure the effectiveness of necessary antibiotic treatments in animals and man.
- To increase the quality of veterinary preventive medicine, and therewith

- To improve the quality of animal husbandry. Therefore a multidisciplinary systems design approach is needed.
- To improve the reputation and societal acceptance of animal husbandry, hence its “Licence to produce”.

Thus, the aim of this White Paper is to support **sustainable disease prevention** to support **sustainable animal production**. Sustainable solutions are characterized by lack of negative evolutionary consequences, long-lasting effectiveness, safety, lack of transfer of negative consequences to future generations or other parties, economic perspectives, and societal acceptability.

This means that solutions have to be acceptable for farmers and veterinarians, and must allow a profitable animal husbandry. These efforts will guarantee the quality of products from this sector. In addition, we like to foster that solutions, including management solutions and preventive treatments, are effective and based on sound scientific evidence, hence that they are **“evidence-based”**.

A major axiom we employ in this White Paper is that diminished use of antibiotics will result in diminished evolutionary force on resistance markers of bacteria, and hence their gradual disappearance. Indeed, resistant bacteria have no competitive advantage in an antibiotic-free environment, and often a disadvantage (Martinez 2008). Whether this is true for all resistance determinants (for example when they are linked to other genes that confer selective advantage), and whether additional measures (such as eradication by therapy or vaccination) are needed to combat resistant bacteria after the use of antibiotics has stopped needs careful attention. Studies have indeed shown that resistance does not disappear always from a population after the antibiotic is no longer used (ASM 2009).

An example is set in human medicine in the Netherlands, where antibiotic use is among the lowest in Europe. This restricted use, together with effective infection prevention strategies, has resulted in low incidences of infections with antibiotic-resistant bacteria compared with other, in particular southern European countries (EARSS 2004). Evidently, however, such results are not easily transferable to the animal husbandry sector with its high concentration of animals, intensive contact structures, and animal movements.

Because the challenges we face are difficult and complex, we consider it important to present an integrated approach consisting of both veterinary, zootechnic, genetic, economic, cultural, and societal approaches. We must therewith keep in mind that our problem-solving efforts must be aimed at the overall system, in which components of the animal husbandry system and related disease prevention function in the context of each other and with other systems, rather than in isolation. The results will contribute to a new and robust animal husbandry in which sustainable disease prevention is an integrated and central activity.

We consider it important that our solutions do not interfere with, or have harmful effects on public health. For example, finding “just another” antibiotic (which could reflect a tremendous scientific achievement) would be a less sustainable contribution, if it would again lead to the development of resistant bacteria and transfer of resistance markers to the human population (if the new antibiotic would be allowed for treating animals anyway).
Our efforts should thus lead to the development of preventive measures and interventions specifically tailored to animals and the veterinary field, and their effects should be long-lasting, and economically and socially acceptable.

Approach: Concept and objectives

The primary objective of this White Paper is to formulate the research needs that may enable primary livestock producers to practice in a way that results in a minimal emergence and prevalence of antibiotic-resistant bacteria through preventing and fighting infections with minimal use of antibiotics. This requires drastic changes in the way of thinking and handling with regard to disease prevention and control, and this research should help to offer the solutions that enable to use less antibiotics, and to overcome the justified and unjustified worries of stakeholders when less antibiotics are to be used.

In achieving this high ambition for such a complex problem, partial solutions will not suffice. Hence, achieving final solutions must comprise both societal, cultural and technical aspects, and therefore require support from an **integrated multidisciplinary research** program. Thus we will provide **technical solutions ("solutions research") enabling behavioural changes** (Fig. 1).

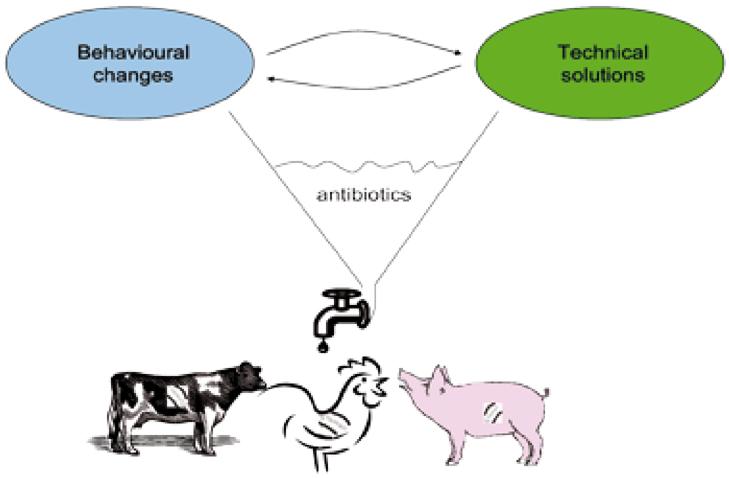

Fig 1. Reduction of antibiotic use in animal husbandry requires behavioral changes of actors in the field, which must be enabled by improved technical solutions. Hence there is a tight relationship between these two.

Goals of the research program are to support:

A. Reduction of the need to use antibiotics in livestock production by eradicating pathogens, hence a **"pathogen-free"** husbandry,

B. To diminish the impact of infections, by designing a **"disease-free"** animal husbandry, where fewer infections occur, and where animals have the genetic, physiological and behavioural possibilities to combat infections with minimal clinical signs and economic losses should infection nonetheless occur.

C. **Reducing, refining, replacing** methods of application of antibiotics to minimize the risk of resistance in bacteria. Therefore research will be directed on understanding the factors that lead to the emergence and maintenance of resistance in bacteria, methods of prudent use of antibiotics that result in fewer treatments and treatments with less risk on resistance (individual treatments in stead of batch treatments, small spectrum antibiotics in stead of broad spectrum antibiotics, parenteral in stead of oral application), and development of diagnostic assays that support such prudent use of antibiotics. In addition it may include the development of alternatives that do not have the disadvantages of current antibiotics.

D. **Implementing changes** resulting in diminished use of antibiotics. Therefore research will be directed on methods of raising awareness and knowledge among farmers, veterinarians, and technical consultants, methods of supporting farmers to reach progress in diminishing the use of antibiotics, methods of benchmarking, formulating guidelines for the prudent use of antibiotics, and governance and behavioural cultural aspects.

E. Development of methods to improve the **monitoring** of resistance. This will provide insight in the effect of measures, and also provide insight into the necessity and strategies needed to combat resistant bacteria that continue to circulate despite the restricted use of antibiotics.

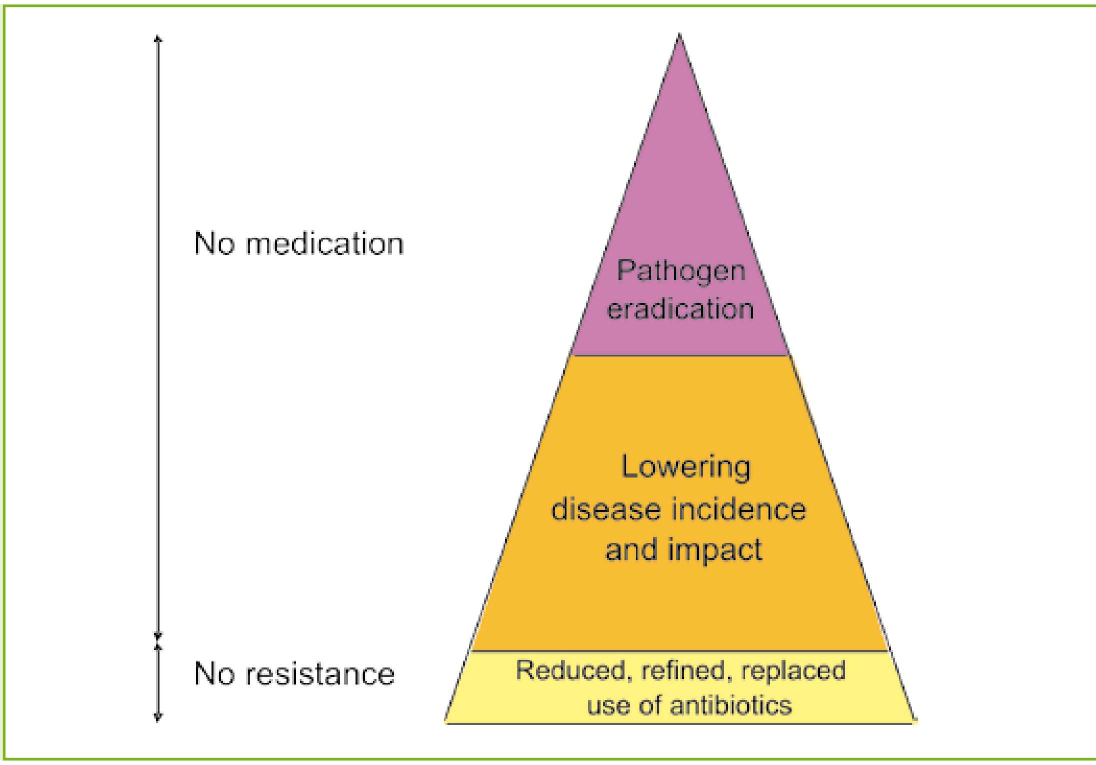

Fig 2. Major conceptual approaches in reducing antibiotic use and resistance are i). to reduce the need to use antibiotics by eradication of pathogens wherever possible and feasible, ii). to lower the incidence, and economic and clinical impact of those infections that cannot be eradicated, and iii). finally by reducing, refining, and replacing the use of antibiotics in such a prudent and responsible manner that emergence and selection of resistance is avoided.

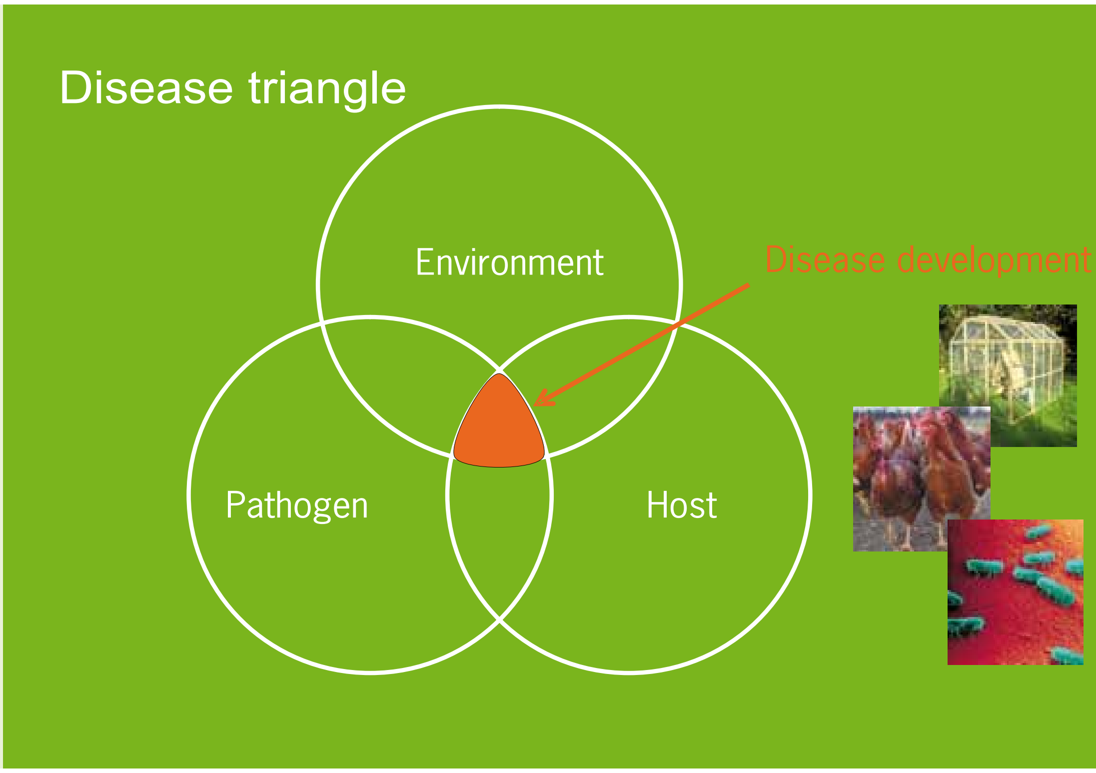

Fig 3. The occurrence of disease is affected by host, pathogen, and environmental factors. Some diseases only occur when unfavorable factors convene.

Progress beyond the state-of-the-art

The research lines described in this White Paper comprise both basic and applied research, all directed at enabling the commercial animal husbandry to ban the systematic use of antibiotics. In several areas this work will increase our understanding of the complex interactions between animals, microbes, and their environment, and optimize the quality with which they are managed.

Although accidental outbreaks of infectious diseases will always occur – no matter how excellent disease prevention works - we consider that the systematic use of antibiotics in commercial farming should be banned as much as possible. Therefore thorough knowledge of host – microbe interactions, factors influencing these interactions, and the ways they can be controlled, is essential so that less of these interactions occur probabilistically. However, also knowledge of managerial, economic and societal factors will be needed to allow systematic reductions in antibiotic use.

We consider it in particular essential that in designing new animal husbandry systems, disease prevention is taken into account. A multidisciplinary field of research that will be stimulated by the research proposed in this White Paper is directed on acquiring a "pathogen-free" status of animal husbandry wherever possible and feasible, hence "eradicology".

If and where pathogen-free working is unreachable, we must try to reduce the impact of infections as much as possible. One of the areas in which significant progress can be made is thus in the "disease-free"[2] design of animal husbandry, intended to optimize interactions between managerial, environmental and genetic factors so that fewer infections will occur and with less impact.

Together, pathogen-free and disease-free design, will not only allow significant improvements in animal husbandry to be made, but will also provide us with in-depth knowledge of microbiological, genetic, physiological and environmental factors, and their modes-of-action, in disease resistance. We thus need to know the contributions of host genetic, pathogen genetic and environmental factors on the variation in disease occurrence.

Much of current antibiotic use is directed at ill-defined enteric conditions that urge us to enhance our understanding of the development of the gut's physiology, including its microbial population. Knowledge thereof will allow us to enhance our knowledge of enteric diseases, and to develop new nutritional concepts that minimize pathogen exposure and colonization, the occurrence of enteric disorders, and suboptimal production levels. Aiming at prevention of antibiotic use, we consider it especially promising to "manage" an optimal composition of the microbial composition in the gut. This may include the deliberate use of beneficial bacteria.

In treating bacterial infections the emergence of antibiotic resistance can be minimized, as well as the effectiveness of therapy improved, if therapy is guided by characterization of the causative microorganisms. This requires knowledge of their virulence markers and sensitivity to antibiotics. Nonetheless, even treatment optimized to the causative microorganism(s) may lead to resistance in the commensal flora, which illustrates that prevention is always superior to treatment. The systematic use of preventive vaccinations (in contrast to reactive or therapeutic vaccinations) of proven effectiveness, for example directed against respiratory diseases, may be optimized by knowledge of the causative strains and their epidemic modes of transmission. This again requires optimization of diagnostic methods to enhance knowledge of causative pathogens and their epidemic modes of transmission. Development of rapid diagnostic techniques is therefore also an important element in this White Paper.

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[2] In this White Paper we use the term "pathogen-free" for an animal husbandry that is free from certain pathogens. "Disease-free" refers to a state where certain microorganisms may be present on farms, but generally without causing (much) disease.

Vaccines are among the most effective means in disease prevention. Both an optimized use of currently available vaccines and newly developed vaccines may therefore significantly help in diminishing the use of antibiotics. In recent years knowledge has been obtained on molecular pathways in the natural immune system that promote and regulate the development of specific immune responses. A great challenge lies ahead to apply this knowledge in vaccine development, especially in the development of adjuvants that help evoking immune responses at mucosal surfaces. While many "easy" vaccines have been developed, vaccines against remaining diseases are, due to various causes, more difficult to develop. We will promote vaccine development against diseases that are responsible for a large antibiotic consumption, for example in treating respiratory disease syndrome in swine, cattle, and poultry, or post weaning diarrhoea/oedema disease in swine.

Also we will contribute to finding alternatives to classical antibiotics although demands for real alternatives are very high. Alternative treatments should not conceal the errors in animal husbandry for which the classical antibiotics may have been used in the past (so we prefer prevention rather than treatment), they should not lead to resistance, especially when they are used for treatment of humans, and they should preferably be broadly applicable. Optimization of phage therapy may be one route and may be achieved in several ways. This approach appears promising in meeting several of these demands.

Finally we will allow making progress in reducing antibiotic use by a true multidisciplinary beta-gamma interaction aimed at optimizing the implementation of both technical and cultural improvements.

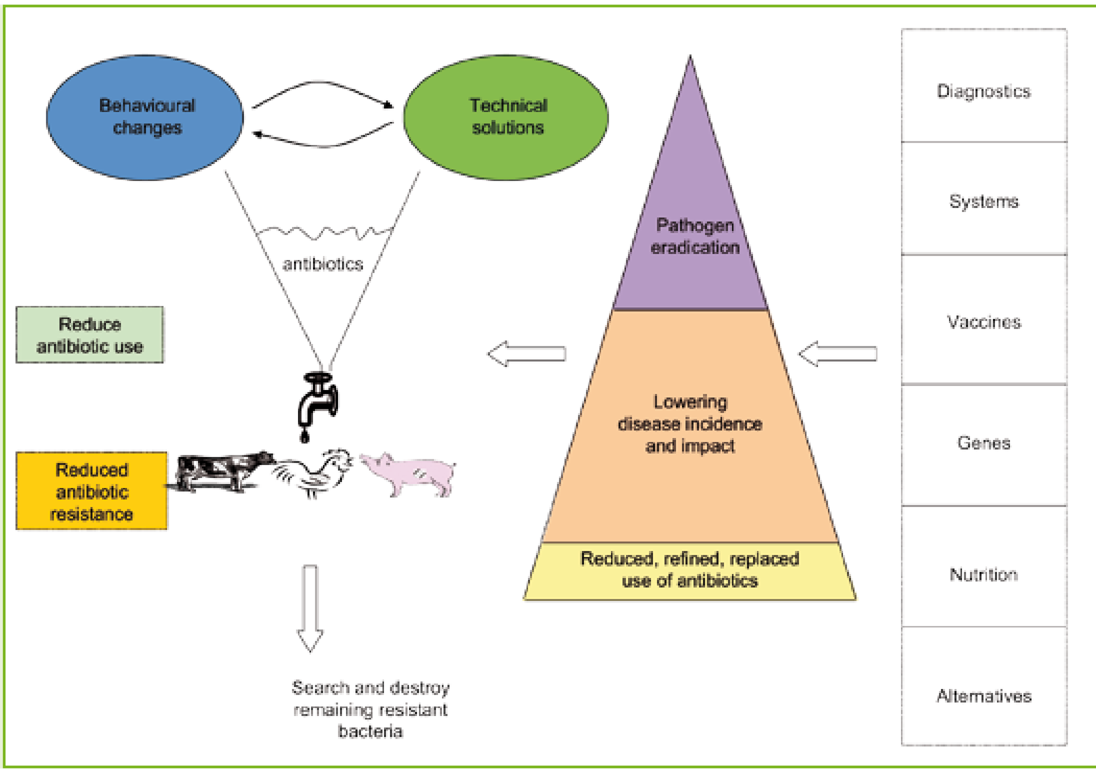


Fig 4. Reduction of antibiotic consumption resulting in diminished selection for resistance can be enabled by pathogen eradication, lowering disease incidence and impact, and finally by reduced, refined, and replaced use of antibiotics. For these goals to be achieved major improvements in the field of diagnostics, husbandry systems, vaccinations, genetic backgrounds of the animals, and nutrition are required. Finally, alternative medicines might be able to replace some of the current antibiotic use.

Work plan

The proposed research will be broken down into work packages that each entails a number of related research questions and employ a set of related scientific and technical approaches. Communication and scientific exchange between the work packages is provided by an overarching work package. We discern the following work packages:

1. Reasons, causes and factors
2. Eradicology: pathogen-free design
3. Disease-free design: lowering the incidence and impact of infections
4. Health-promoting nutrition
5. Better diagnostics
6. Better vaccines and vaccinations
7. Alternatives
8. Implementing changes
9. Registration and monitoring of antibiotic usage and resistance
10. Communication and scientific exchange

Work package descriptions

1. Reasons, causes, and factors

The occurrence of disease is a constant risk in the livestock production sector. Disease can have a significant impact on the production process. The development of the intensive livestock sector benefited a lot from the availability of antibiotics. Antibiotics facilitated the work of veterinarian and farmer. They were effective, reliable and reasonably cheap. This made antibiotics the first choice in the treatment of animal diseases, either because the disease was caused by susceptible bacteria, or because the use of antibiotics could prevent secondary infections. It even turned out that the use of antibiotics could prevent the occurrence of these primary and secondary infections.

All this has led to an excessive use of antibiotics and cushioned the need for the development of alternatives either in treatment or prevention. The widespread occurrence of antibiotic resistance was an unanticipated side-effect and forces the intensive livestock sector to reconsider the use of antibiotics as panacea for all disease problems.

Until recently there where not so many incentives for farmers and veterinarians to restrict the use of antibiotics. A major incentive was to avoid residues in the products. In the sectors dairy production and egg production with its continuous production of end-product, the use of antibiotics is much lower than in other livestock sectors that produce meat. Since the majority of the disease problems in these sectors occur in the beginning of the production cycle residues are hardly a problem.

Research in this work package is directed on the reasons, causes, and factors that urge farmers and veterinarians to use antibiotics, but also on the epidemiological factors that determine the selection and spread of resistant microbes and of resistance determinants. This work will be analytical and therefore provide us with detailed knowledge of the indications and causes for which antibiotics are used, and the mechanisms that lead to antibiotic resistance. The subsequent work packages will build on this knowledge and will be problem-solving.

The aim of the research in this work package should focus on identifying the sources of antibiotic resistance to direct prevention and control strategies to these sources. This is evidently a broad area of research ranging from basic insight in diseases, causative microorganisms, and host-microbe interactions to managerial, economic and societal aspects of disease and antibiotic usage. Research in this area requires careful definition of in- and exclusion criteria of study subjects, hypotheses, and research approaches. As there are large differences both between countries and individual farms in their use of antibiotics, it is indicated to examine the reasons for this variation and to indicate risk factors for antibiotic use and the occurrence and spread of infections and resistance markers. This will provide us with knowledge of the "best practices" available at present.

Furthermore research in this work package will provide us with the knowledge and tools whereupon interventions, to be worked out in the following work packages, may be based. The same holds true for the development of measurable parameters to monitor progress in this field. Model approaches may be helpful in structuring the thinking and allowing the evaluation of alternative interventions and combinations thereof.

For the sake of discussion, we may divide reasons for antibiotic use as follows:

1. Known diseases with a monocausal etiology
2. Known diseases with a complex multicausal etiology
3. Unknown or insufficiently characterized and ill-understood disorders.
4. Management insufficiencies, probably including a range of –implicit or explicit- reasons, such as putative growth promotion, insufficient disease prevention (no or inadequate vaccinations), insufficient quality of food and housing (incl. ventilation), good economic experiences, insufficient time and efforts for proper diagnosis and other interventions, routine, ease, for the sake of safety, and as insurance.

Known diseases with a monocausal or complex multicausal etiology

The use of antibiotics in animal production is for a significant part related to disorders of the respiratory tract (caused by a range of pathogens, including parainfluenza virus type 3 (PI-3 virus), bovine respiratory syncytial virus (BRSV), *Pasteurella* and *Mannheima* spp. in calves, porcine respiratory and reproductive syndrome virus (PRRSV), influenza virus, *Mycoplasma* spp. in pigs, and disorders of the gut, such as post-weaning diarrhoea (colibacillosis), necroproliferative enteritis (or porcine intestinal adenomatosis [PIA], caused by *Lawsonia intracellularis*), dysentery (*Brachyspira hyodysenteriae*) in pigs, and necrotic enteritis (*Clostridium perfringens*), malabsorption syndrome, coccidiosis, and chronic enteritis in poultry.

These diseases may be monocausal or they may have a complex etiology, for example a primary viral infection followed by bacterial superinfection, or an infectious etiology aggravated or enabled by poor environmental factors.

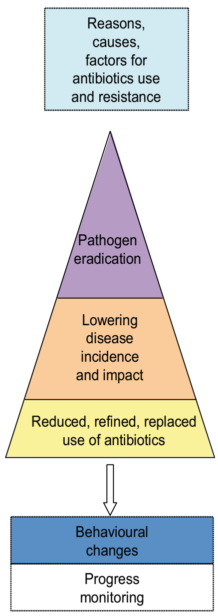


Fig 5. Rational interventions aimed at reducing antibiotic use start with acquiring knowledge of the reasons, causes, and factors for antibiotic use and resistance.

There is also a number of important systemic diseases in (young) pigs, such as *Streptococcus suis*, which may cause a high disease rate, mortality and decreased well-being. S. suis infections are normally treated with antibiotics. In 2006, it was found that 50% of the use of antibiotics in weaned piglets in The Netherlands was related to treatment of S. suis.

It is noteworthy that known respiratory tract pathogens in swine and calves are frequently occurring in many herds, but do not always result in disease. This may indicate that data from limited diagnostic efforts may be over-interpreted and used as evidence for a causal aetiology, thus providing a justification for treatment. Examples are misinterpretation of (single) serological analyses or over-interpretation of PCR assay results without further clinical or pathological support.

Identifying and monitoring the causative microorganisms of these known diseases should be directed on detecting trends in occurrence, and especially on detecting changes in virulence and antigenic composition that may interfere with vaccination. Research may further be directed on the epidemiological and evolutionary mechanisms that determine the occurrence and severity of endemic infections, including those of resistant bacteria.

Such work will also provide answers to the question whether resistant bacteria will cease to spread after the use of antibiotics as an evolutionary force has stopped or diminished drastically. For example, studies indicated that the use of standard antimicrobial medication of the pigs appeared to be a clear risk factor for MRSA carriage (Van Duijkeren et al 2008). Diminished occurrence of vancomycin resistance of human enteroccooi has been observed after the ban on avoparcin (which resembles vanocmycin) in animals, but the question is whether such experiences are generally true for all antibiotic-resistance marker combinations. (Note that the occurrence and increase of vancomycin-resistant enterococci in human medicine was probably also the result of avoparcin use as growth promoter in livestock).

Other research is aimed at the question which antibiotic use (types, routes, doses, etc), together with co-factors (disinfection procedures, biosecurity between herds and during transport of animals) or not, predominantly contributes to the emergence, spread, and maintenance of resistant bacteria. This may contribute to optimized use of antibiotics.

While these diseases and pathogens are more or less well known, research may be restricted to:

- Establishing and verifying which diseases and pathogens are responsible for most antibiotic use.
- Monitoring temporal changes in the occurrence and characteristics of causative pathogens, in particular their potential changes of virulence markers and antigenic composition that may hamper vaccine effectiveness.
- Defining the epidemiological, evolutionary, and pathophysiological mechanisms that determine the occurrence and severity of endemic infections, including those of resistant bacteria.
- Identifying which factors (including aspects of antibiotic use) predominantly lead to emergence, spread and possible maintenance of resistant bacteria and resistance markers.
- Establishing the role of animal movements in the production chains (i.e. the pyramid structure) and management factors that affect infectious pressure and spread of resistant bacteria and resistance markers.

Unknown or insufficiently characterized and ill-understood disorders

As mentioned, the majority of antibiotic use in animals is used in so-called "herd or batch treatments", and much of these antibiotics are used for treating or preventing respiratory tract infections of unknown aetiology and non-specific enteric disorders. Interestingly, while the deeper respiratory tract is largely a "sterile' organ, the enteric tract normally contains a huge population of microbes interacting with each other and their host.

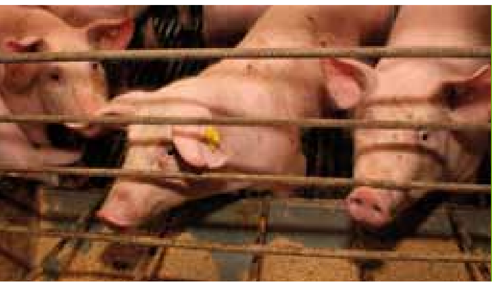
Non-specific or insufficiently characterized enteric disorders probably comprise so-called dysbacterioses, or unbalanced microbial populations in the gut. This condition is thought to reflect an overgrowth with certain pathogenic species of bacteria (as cause or consequence). It can originate (1) from, or be caused by an insufficient development of the enteric immune system, or (2) antibiotic treatments. A comparable condition in humans is known as "small intestinal bacterial overgrowth" and is thought to be secondary to underlying predisposing disturbances (Abu-Shanab and Quigley 2009). One of the bacterial species that might colonize in such circumstances is *Clostridium difficile*. Its pathogenic significance in farm animals is probably small (only in pigs), but this species is pathogenic for man, and is often resistant to a range of antimicrobial agents, including the fluoroquinolones (Coja 2009).

These ailments thus appear ill-defined, both in veal calves, swine, and poultry. Note also that the causative role of some well-known microorganisms in disease, including *Ornithobacterium rhinotracheale, Enterococcus caecorum* and *Enterococcus hirae* in poultry, and porcine circovirus type 2 (PCV2) in swine, is insufficiently clear.

There are also indications that animals living in suboptimal health conditions, challenged with pathogens and with an activated immune system, have different nutrient requirements than animals living in a more clean environment. This concerns for example the requirement for essential amino acids. It is unknown whether such animals may be more susceptible to infection. Antibiotics may be used to inhibit subclinical infections associated with metabolic costs of activation of the natural immune system in such animals. More quantitative information therefore needs become available on nutrient requirements of farm animals and on the consequences of insufficient nutrition on disease occurrence, so that diets can be adjusted to give maximum support to the animal's requirements and defence mechanisms.

Altogether it appears that the optimal development of the gut's function, including the development of its immune function and microbial population, is not well known, as are disturbances of these functions and their relationship with disease.
Evidently when studying these diseases, it is indicated to try and find the – preferably single - cause of the diseases, or – in the case of complex disorders – to try and define the relative contributions of pathogens and environmental or managerial risk factors.

Moreover, as insufficiently characterized disorders may be difficult to reproduce under experimental conditions, it is required to use strict research protocols under field conditions, to work hypothesis-driven where possible, to establish causative relationships were possible, and to formulate "a golden standard" for diagnosis. In conclusion, it is indicated to:



- Characterize ill-defined disorders with respect to aetiology, pathophysiological mechanisms, and causative (co-)factors. Define them precisely.
- Such research should especially be directed on finding clues for preventive measures. Measures have to be evaluated on effectiveness, cost benefit ratio, ease to implement, and reliability.

Management insufficiencies
There are large differences between farms in antibiotic use. For example, at broiler farms the 25 % major users account for approximately half of the total antibiotic use (MARAN 2007). The reasons for these differences are largely unknown, but may include aspects of hygiene, management, and feed quality. The reasons for these differences have not been thoroughly investigated until this far.

In searching for management characteristics related to the use of antibiotics, it is important to note that a number of animal owners really take on a role as forerunner in reducing antibiotic use, and manage to do so with and without professional support. Knowledge of reasons for qualified and non-qualified use will likely result in clues for improving management, lowering antibiotic use, and ways to support farmers in reducing antibiotic use. Peer groups of farmers working on restriction of antibiotic use and learning from each others' best practices may therefore be able to significantly reduce the use of antibiotics on their farms. Therefore research should include to:

- Establish the reasons (diagnoses, arguments, incentives, management disorders) for antibacterial use. Therewith define the major reasons for farmers and veterinarians to decide to treat animals with antibiotics.
- Examine differences between farmers in antibacterial use. Therewith take socio-economic, behavioural, and attitudinal aspects that may determine antibiotic use into account.
- Relate management factors (e.g. housing, group size, climate control, import of animals, all-in all-out systems, age and method of weaning, nutrition, behavioural factors etc) with the quantity of antibiotic drug use on farms, explain differences between farms, and define "best practices" for a sparse use of antibiotics.

2. Pathogen-free design

When many animals are held closely together, pathogens have high chances on transmission, they may multiply to high numbers, and they may so optimally express their disease-causing abilities.

Pathogen eradication or a pathogen-free design of animal husbandry is considered in this White Paper as one of the main options to control disease provided that a stable and sustainable pathogen-free status may be reached and maintained at reasonable costs and without unwanted disadvantages.

Pathogen-free design or eradication may be aimed at global, regional, or herd levels. Successful examples include the worldwide eradication of smallpox and the regional eradication of polio in humans, and the country-wide eradication of pseudorabies virus and at herd scale of diseases such as PRRSV in animals.

Experience has also taught less successful examples and hurdles, for example setbacks in the eradication of polio due to live vaccine reverting to virulence, or of PRRSV due to insufficient effectiveness of vaccine. Eradication of *Mycoplasma hyopneumoniae* at pig herd level based on age-segregation and medication has proven possible, but with a permanent risk for re-infections (Maes et al 2008).

To further optimize the disease status of animal husbandry and to lower antibiotic consumption we consider it important to use the principle of pathogen eradication and, or pathogen-free designed production more generally in livestock production, i.e. wherever possible and feasible. Challenge is to implement this in the dense livestock industry in The Netherlands. Aspects of the eradication or pathogen-free approach are currently used, among others, in SPF farms and High Health farms. Evidently, there is a different situation when "pathogen-free" herds (such as SPF or High Health) can be established from the start, while conventional farms may first need specific eradication efforts to become free of certain pathogens. In the end both types of farms need similar strategies to remain pathogen-free.

Requirements for pathogen eradication in general are more or less known, and include the availability of sensitive diagnostics to detect the spread of infection and the detection of carrier animals, high-quality intervention methods to interrupt pathogen transmission, strategies as depopulation-repopulation or pathogen elimination by vaccination, the lack of a reservoir or carrier animals from which the pathogen may be reintroduced, and (preferably non draconic) biosafety measures of proven effectiveness to prevent reintroduction of the pathogen including a regulated animal flow (Needham and Canning 2003). If either the rate of introductions or the costs of fighting outbreaks upon (re-)introduction are too high, eradication may not be the best option. Evidently this requires diagnostic and intervention tools of high quality, as well as thorough knowledge of the population dynamics of pathogens to allow interrupting transmission chains of pathogenic microorganisms based on scientifically sound cost-benefit analyses.

We consider it important to develop a sound and firm theoretical framework underlying a pathogen-free animal husbandry. This means that pathogen-free design should be directed on the robust performance of farms and the farming system as a whole. Therefore a multidisciplinary systems design approach is needed with contributions from amongst others microbiology, zootechnics, genomics, systems biology, ecology, and risk assessment studies. Evidently, demands with regards to pathogen eradication or pathogen management need to be reconciled with demands on animal welfare, sustainability, and economics.

Major issues are whether pathogen-free design and pathogen eradication should be directed on a pathogen-for-pathogen base, or whether they should be directed on groups of pathogens simultaneously, and what measures should be taken in the case of accidental reintroductions. A microbiological risk categorization of farms may be useful in acquiring and maintaining a pathogen-free status for specifically defined groups of pathogens. Attention should be given to the different strategies that may be needed on new farms, and on existing conventional farms. Likewise, attention should be given on how networks or chains of pathogen-free farms may optimally function. Although the focus should primarily be directed on acquiring and maintaining a pathogen-free status of farms for defined groups of pathogens, "pathogen-management" may also be useful to lower the infectious pressure on farms when a pathogen-free status is temporary lost or unachievable. Deliberate introduction of "beneficial bacteria" may be useful to optimize the functioning of the immune apparatus and gastrointestinal tract.

Note also that pathogen eradication strategies may be feasible or required to combat remaining resistant bacteria, such as MRSA, after the use of antibiotics has been diminished.

To facilitate this strategy the following activities are considered relevant:

- Develop a theoretical framework employing a multidisciplinary systems design approach that may underlie a pathogen-free animal husbandry.
- Provide a comprehensive overview, employing risk analysis, knowledge of population dynamics of endemic pathogens, and cost-effectiveness calculations, of the pathogens for which eradication at herd or regional level may be feasible at a wide scale. Examples may include PRRSV, PCV2, *Actinobacillus pleuropneumoniae, Haemophilus parasuis, Mycoplasma* spp., *Pasteurella multocida, Bordetella bronchiseptica, Brachyspira hyodystenteriae, Lawsonia intracellularis,*

Streptococcus suis (pigs), *Salmonella gallinarum, Mycoplasma gallisepticum, Ornithobacterium rhinotracheale,* and *Brachyspira* spp. (chickens).

- Formulate preconditions for eradication of selected pathogens at herd level.
- Formulate how the structure of the animal production sector and internal management systems at farms should be employed or optimized to support and maintain pathogen eradication.
- Optimize methods of eradication and provide evidence of their effectiveness.
- Perform feasibility studies to eradicate selected pathogens on a wide scale at herd level.
- Consider the possible need to eradicate resistant bacteria.
- Formulate requirements for supportive diagnostics and vaccines.

3. Disease-free design: lowering the incidence and impact of infections

When it is for - certain pathogens or certain groups of pathogens – not feasible to make and keep farms free from them, we should try to reduce their infectious pressure and keep the clinical and economic impact of infections as low as possible. This is also referred to as "elimination of disease". Evidently animals that experience less clinical symptoms upon infection will require less antibiotics.

According to the "iceberg concept" of infectious diseases, many infections run a subclinical course (Fig. 6). Clearly, the genetic make-up of the animals, the genetic properties of the endemically circulating pathogens, the environment in which the animals are living, and their mutual interactions determine the number and severity of infections the animals experience, and hence the impact of infections on the animals. For example, interactions between infected and non-infected hosts determine the rate of infection or infectious pressure. It is also well known that stress periods, such as early weaning of pigs and early lactation of cows in a negative energy status, make animals more prone to infection. We will thus try not only to reduce the number of infections, but also to let them more often run a subclinical or less severe course.

The environment

The environment of the animals includes a number of aspects (housing, group size, climate control, restricted import of animals, all-in all-out systems, weaning age and method, vaccination schedules, controlled exposure to pathogens, SPF status, nutritional status) that

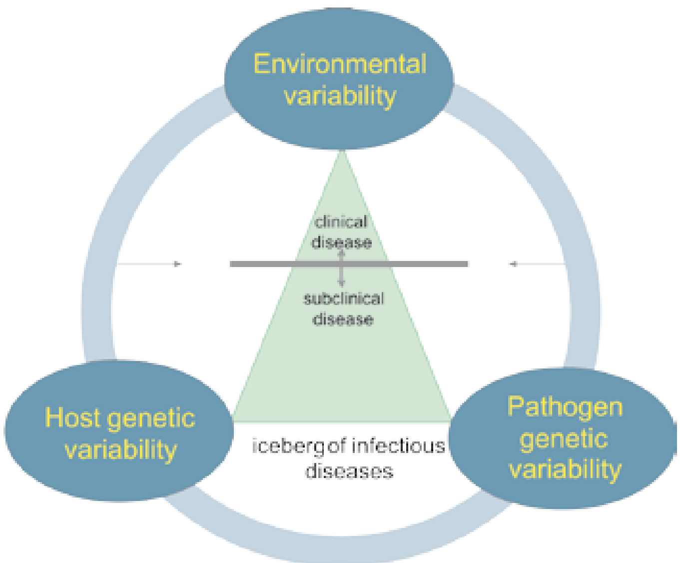


Fig 6. The ratio between infections that run a clinical or a subclinical course is affected by variation in host genes, variation in microbial genes, environmental variability, and their often complex interactions. Optimization of these factors may diminish the incidence of infections, as well as their clinical and economic impact.

may affect health status. Such environmental factors may both affect the chance of acquiring infection (animal-to-animal transmission of pathogens), as well as the animal's ability to resist or to cope with the infection. Therewith come opportunities to manage the exposure of animals to pathogens.

It may – for example – be tempting to try to manage the time and dosing of infectious encounters. In a natural environment young animals develop lasting protection if they encounter pathogens in low numbers while under protection of maternal antibodies. It is not yet clear how much of this natural mechanism can be utilised in the environment of a production system, but it is worth exploring it for endemic pathogens.

Likewise, a large proportion of the antibiotics used (in number of dayly dosages per animal) is supplied after an accumulation of changes that the animal perceives as stressful, the so-called transition periods. In many cases, animals are exposed to new, sometimes opportunistic, pathogens in this vulnerable state. Weaning pigs is an obvious example of this situation. One research objective is to re-design production systems and their management to avoid accumulations of stressful events that affect a large number of animals simultaneously by exposing them to a new ecosystem of micro-biota (i.e. re-location to another pen). Alternatively, we may develop specific protection methods during these transition periods. Methods may include teaching young piglets to eat solid food before weaning in order to prevent weaning diarrhoea, or to modify the nutrition of cows in early lactation to prevent metabolic disorders and associated infections.

So far, most of the genetic and environmental factors have been optimized - in an implicit or explicit way- to meet economic demands. Now it is thus important to develop methods aimed at optimizing infectious disease resistance in the absence of antibiotics, and to understand the scientific base thereof. It is also important to take the sometimes complex interactions between genetic and environmental factors into account. Furthermore we consider it important that methods and criteria will be developed to demonstrate the effectiveness, both in relation to economic results and disease management, of proposed changes in management and genetic selection. Hence that they are **“evidence-based”**.

Research in this work package will be directed, first, on the development of husbandry systems that limit the occurrence and spread of specific infections wherever possible (also in relation to the previous work package), and, second, on minimization of clinical symptoms and economic consequences where the occurrence of infections cannot be prevented, thereby taking into account the relationships and trade-offs between defence mechanisms and productive functions. Proper vaccination programs are an integral part of such husbandry systems (see WP 6). Thus we may take several approaches, but taking great care that in the end they will lead to integral solutions, i.e. an optimized system design. This will be done in close collaboration with WP 2. The approach is to:

- Understand and optimize management factors that may lower the infectious pressure on farms, or that may optimize the animal's resistance and resilience to infection, in particular during stressful events.
- Identify and study immune modulatory effects that are affected by management measures and that influence infectious disease resistance.
- Define stressful transition periods during the production cycle during which the animals are most susceptible for disease development (e.g. weaning, transport), identify the causative pathophysiologic mechanisms, and develop methods to prevent disturbances of homeostasis, and/or to support and improve the relevant biological processes (e.g. the application of specific immune modulators (pre-/ probiotics) during weaning).

- Explore the ability of young animals (protected by maternal antibodies) to develop long lasting protection after exposure to low numbers of pathogens.
- Provide evidence of the effectiveness of management interventions and new system designs.

The genes

In addition to the influence of environmental and physiological influences, there is ample evidence of genetic variation in susceptibility to specific diseases. Although a relatively new field of research, polymorphisms in a number of host genes in several species have been documented that affect infectious disease resistance.

Such genes may for example affect the natural resistance of tissues to infection, the inflammatory response to infection, the natural and the acquired arms of the immune system, antigen presentation, etc, etc. Genetic selection thus creates an advantage or disadvantage in resisting the establishment of infection or expressing clinical symptoms.

In exceptional cases genetic resistance to infection is based on one single gene, but for most infections genetic resistance is complex and based on several genes (Kimman 2001). So far there has been little successful utilization of gene-based selection for disease resistance. Scrapie in sheep is one successful example, but another nearby example may be selection of pigs for resistance against *E. Coli* F4 based on the MUC4 gene (Peng et al 2007, Rasschaert et al 2007, Wang et al 2007).

In contrast to the clear evidence that the host's genetic make-up may provide varying degrees of resistance to particular pathogens, there is up to now little to no evidence of genetic variation in susceptibility to disease in general, resilience to many infections, or in general immuno-competence. Indications that resistance may be broader are the occurrence of bovine lines with lower susceptibility to clinical mastitis, and chicken lines with higher resistance against Gram-negative bacteria. A trade-off of genetic resistance, might however be lower productivity. Nonetheless, in addition to genetic selection for specific pathogens, genetic selection in a target environment may be a meaningful way to increase the natural or general defence mechanisms to the various infectious pathogens to which the animals are exposed "in daily live".

Altogether, recently developed methods, such as genomic selection, open up new opportunities to select against susceptibility for diseases. However, one evident difficulty in studying and exploiting genetic disease resistance is that phenotypic expression of infectious disease resistance can only be established after infection, either experimental or natural, has taken place. That leaves two approaches, either studying and using resistance upon experimental infection of genetically good characterized animals, or studying and exploiting genetic resistance upon natural occurring infections. The exposure of the animals to such natural infections has to be established, and "proof of principle" of both approaches should be provided. Finally, complex interactions between host genetic, pathogen genetic and environmental factors should be taken into account. The objectives are:

- To provide further proof of principle of genetic approaches to enhance disease resistance.
- To integrate genetic and pathophysiological data on particular infectious diseases to identify relevant genes, gene variants and molecular pathways that can be used as a target to improve animal health by genetic as well as non-genetic approaches.
- To establish host gene-pathogen gene-environment interactions.

- To increase our understanding of the various molecular genetic pathways that animals use to resist establishment of infection or to avoid developing clinical symptoms by comparing high and low susceptible selection lines by functional genomic approaches.
- To elaborate on the new tools of genomic selection (including 60.000 SNP chips) to develop practical ways to select against disease susceptibility and the development of clinical symptoms upon infection.
- To improve the accuracy of genomic breeding value predictions with respect to phenotypes related to infectious disease and the use of antibiotics.
- To develop an animal trait ontology in the field of livestock infectious diseases.

4. Health-promoting nutrition

It is suggested that part of the antibiotic use in animal husbandry may be explained by an inadequate microbial population in the gut, and, or imbalances in the supply of nutrients. The gut hosts a complex microflora, which plays a role in the prevention of colonization of pathogens, regulation of tolerance and immune responses, and digestion and absorption of nutrients. Disturbances in the normal flora and the physiology of the gut may therefore lead to colonization with pathogenic microorganisms, reduced digestive activity of the gut, and enhanced translocation of pathogens through the gut due to disturbances in the integrity of the epithelial barrier or to improper functioning of innate immune mechanisms. Such disturbances may occur during periods when demands on feeding and nutrient provision are very high, the animals experience stress, and large changes in the gut physiology occur, i.e. the immediate post-natal and post–weaning periods.

Disturbance of energy homeostasis in the gut may for example lead to increased intestinal permeability and increased oral *Salmonella* infection (Rodenburg et al 2008). This implies that strategies to reduce infection may include management for proper intestinal (energy) homeostasis. This requires understanding the relationship between intestinal energy metabolism and disease resistance, and defining thresholds for the intestinal capacity for nutrient and energy management related to animal growth and development.

Feed components, such as additives, feed acids, feed salts, anti-oxidants, pre- and probiotics (including lactobacilli, lactococci, etc), digestive enzymes, lactoferrin, anti-inflammatory treatments may restore or prevent disturbance of microbial equilibriums in the gut, or the ability of the animal to cope with them.

Research in this work package is directed towards identifying factors that may optimize microbial equilibriums, intestinal immunity, and the efficacy of feed digestion, as well as their modes of action. Major emphasis will be placed on developing models and measurable parameters that allow to predict health effects, and on methods of managing the microbial population of the gut. One promising approach may be the optimization of probiotics as a tool in the prevention of enteric disorders.

Probiotics are non-pathogenic bacteria that compete with or interfere with the colonization of pathogenic bacteria and promote the development of the normal function of the gut. Their modes of action are only incompletely known, and may involve changes of the gut milieu, release of bacteriocines, competitive exclusion of pathogens, and stimulation of the natural immune system in the gut. In poultry the use of probiotics, based on complete gut flora, is already known as "starter cultures" (Schneitz 2005).

Measuring the effects of nutrition and feed ingredients

A number of feed additives are currently on the market which directly or indirectly affect the function of the gut and microbial homeostasis in the gut. According to the current EU regulations (Regulation (EC) No 1831/2003) feed additives are classified into the following

categories:
- Technological additives (e.g. preservatives, antioxidants, emulsifiers, stabilising agents, acidity regulators, silage additives).
- Sensory additives (e.g. flavours, colorants).
- Nutritional additives (e.g. vitamins, minerals, free amino acids, trace elements).
- Zootechnical additives (e.g. digestibility enhancers (enzymes), gut flora stabilizers (pre- and probiotics), and
- Coccidiostats and histomonostats. It should be mentioned that a large number of feed additives do have more than a single effect when present in the diet.

Feed may also contain natural constituents that are more resistant to enzymatic or fermentative degradation, which could have beneficial functional properties. These could influence intestinal passage rate and might serve as e.g. adhesive for pathogenic microbial species, thereby preventing the colonisation of the gastrointestinal tract with pathogens. Besides, the feed ingredients could contain small fractions of constituents with bioactive capacities, such as lactoferrin and lactoperoxidase or antimicrobial proteins.

Other feed ingredients which have been investigated for their functional properties are linseed meal, oats (Jansman et al., 2006), legume seeds (Jansman 2007, Jansman and van der Meulen 2008). There is, however, still a scarcity of knowledge on the functional properties of feed ingredients in relation to disease prevention in terms of their identification and application under defined conditions. Some examples are given below.

Functional properties of feed ingredients in relation to disease prevention

Fraction	Effects
Fermentative constituents	Composition and activity intestinal microflora
Adhesive constituents	Composition intestinal microflora, colonization pathogens
Antimicrobial peptides and proteins	Composition intestinal microflora, colonization pathogens
Gel forming carbohydrates	Protection of intestinal mucosa
Other bioactive compounds	Antimicrobial effects, effects on the immune system

Research should be directed into the effectiveness and precise mode of action of feed ingredients, additives, and their potential functional constituents. This research should include effects on resistance to specific pathogens, on the commensal microflora in the gut, on the host's immune system, and on the overall effects on health and performance. Therefore the development of predictive animal models is of utmost importance.

Major emphasis should be placed on developing measurable parameters that indicate gut function and microbial homeostasis, and hence antibiotic use, and on managing the microbial population of the gut. It is therewith of utmost importance that claims are substantiated in good experimental infection models and under field circumstances. Therefore we consider the following activities important:

- Development and application of experimental infection models, either using mono- or multi-infections, in which the influence of nutritional factors on clinical and microbiological outcome parameters of infection, and their modes of action, can be examined.
- Understanding of the mechanisms by which feed constituents affect infectious diseases.
- Development of measurable parameters of gut function and microbial homeostasis.
- Development and application of field study protocols, for example guided by principles as used in randomized, double-blind, placebo-controlled and cross-over studies, to unequivocally allow demonstrating the effect of nutritional factors on clinical, microbiological, and economic parameters under field circumstances.

Understanding the effects of nutrition and feed ingredients on gut health

Only now we have the necessary technological advances and ecological concepts at hand to define and understand modes of host-microbe and nutrient-host interactions, and their impact on host health (Ley et al., 2006). Colonisation of the intestinal epithelium was found to trigger the expression of host genes involved in intestinal maturation, blood-vessel formation, mucosal barrier fortification and development of the immune system (Hooper, 2004, Turnbaugh et al., 2006).

Most importantly, commensals 'collaborate' with the host to produce bactericidal molecules that help to prevent excessive colonisation by unwanted and potentially pathogenic microorganisms. The study of these interactions may be called "gut ecology". The intestine remains relatively immature and permeable to antigens during the first days of life (Petersen et al. 2003; Marion et al. 2005). Microbial colonisation has been shown to modulate maturation postnatally and after weaning (Boudry et al. 2004; Montagne et al. 2007, Chowdhurry et al. 2007). More specifically, it could be demonstrated that microbial flora induces i) the expression of genes contributing to intestinal epithelial cell turnover and mucus biosynthesis, and ii) immune responses in order to prevent inflammatory responses that would compromise barrier function (Calder et al. 2006).

Nutrigenomics is aiming to understand how feed (nutrients and other components) affects gene expression, and how genomic differences affect the way animals respond to nutrients. However, the mechanisms by which nutrients specifically regulate the expression of genes is so far poorly understood. Recently genome-wide approaches have become available to study one aspect of the regulation of gene function, i.e. epigenetics (Fig. 7). Epigenetic changes are heritable changes in DNA that do not change the sequence of the DNA, but do influence gene expression. Two types of epigenetic changes are methylation of DNA and modification of histons (Fig. 8).

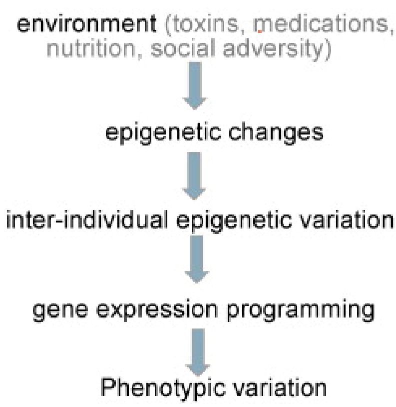
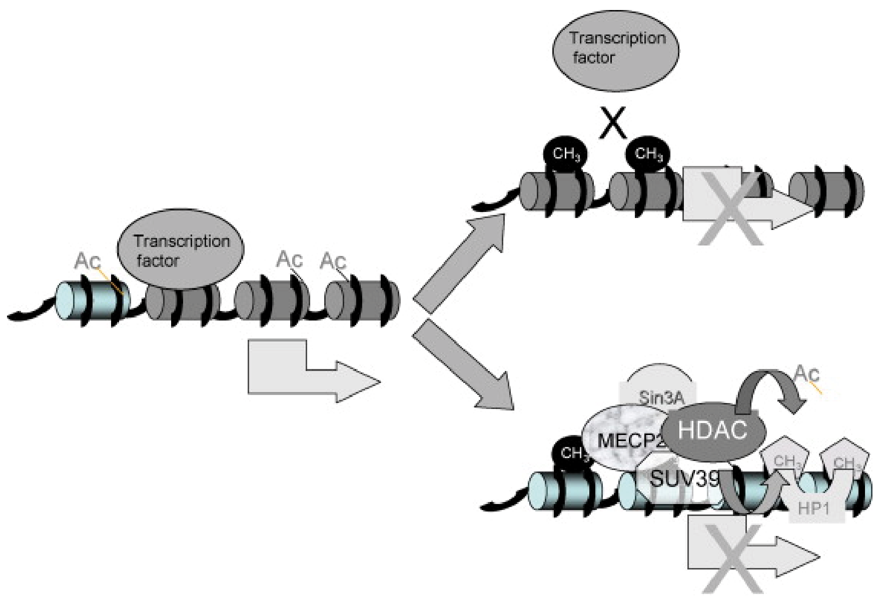


Fig. 7. DNA mehtylation is a reversible process targeted to cytosines in DNA, mainly takes place in promoter regions containing CpG dinucleotides, and results in repression of transcription.

Fig. 8. Two mechanisms to repress gene expression by DNA methylation: i). Methylation of cytosines prevents the binding of transcription factors to DNA thus inhibiting transcription (above), ii). Methylated DNA Binding Protein (MECP2) binds to methylated DNA, thus enabling the binding of other transcription repressors (below).



There is vast literature on the relationship between nutrition and epigenetic changes, in particular during development stages. Methyl donors in the diet, such as folic acid, vitamin B12, methionin and cholin, appear relevant in epigenetic regulation. However, relatively little is known about the effects of diet composition and nutrition on epigenetic changes in farm animals. These changes are likely to exist and may be relevant in relation to disease resistance.

Understanding the gut's physiology and its relation to microbial colonisation of the gastrointestinal tract may be powerful tools to strengthen intestinal barrier function and immunological competence of young animals. Research emphasis in this area should be on:

- Providing a sound understanding of gut function and its interaction with bacterial colonisation by normal and pathogenic microbial flora, particularly in young animals and especially around transition periods (e.g. the time of weaning).
- Identifying improved feeding strategies and bioactives, and their modes of action, as alternatives to the use of antibacterial medicines as prophylactic treatments and growth promoters.

Steering the gut's functions

Based on a sound understanding of the gut's physiology ways to positively influence the gut's function may be developed. A novel way of controlling pathogens may be to manage the micro-biota in the gut to avoid major disruptions of the micro-biota ecosystem. Therefore we must develop skills to monitor and manage the balance between pathogenic and non-pathogenic micro-organisms.

For example, it is becoming clear that the cationic antimicrobial peptides are an important component of the innate defences. Such peptides can be constitutively expressed or induced by bacteria. The best peptides have activities against a broad range of bacterial strains, including antibiotic-resistant isolates. They kill very rapidly, do not easily select resistant mutants, are synergistic with conventional antibiotics, other peptides, and lysozyme, and are able to kill bacteria in animal models (Byrne et al., 2007). Antimicrobial proteins are present at the intestinal epithelial surface and serve as another innate defense mechanism (Brisbin et al., 2008, Moal and Servin, 2006).

One category of antimicrobial peptides, defensins, are cationic proteins that function by causing cell lysis. Three subfamilies of defensins exist, α-, β- and γ-defensins. To date, 13 avian β-defensins, also called gallinacins have been described (Brisbin et al., 2008, Ganz, 2003). Gallinacins are important innate defense proteins in the chicken GALT with potent activity against intestinal pathogens (Hasenstein et al., 2006; van Dijk et al., 2007). These authors observed an increase in the expression of several gallinacins following *Salmonella* infection in chickens. Importantly, administration of probiotics prior to inoculation with *Salmonella* resulted in a decrease in the expression of the genes that encode certain gallinacins.

Cathelicidins, another family of antimicrobial peptides with broadspectrum antimicrobial activity, have also been identified in the chicken. Host defence proteins can be either constitutively expressed or induced in response to specific stressors such as infection and inflammation. α-defensins tend to be produced constitutively, whereas the majority of ß-defensins are inducible. Moreover, α-defensins have evolved to operate mainly from within phagosomes, whereas ß-defensins are produced primarily by epithelial cells (Linde et al., 2008).

Research should be performed on how the expression of these endogenous proteins and peptides can be stimulated in a balanced way, and so contribute to (natural) disease resistance. A promising approach in this respect may be the optimization of probiotics, for example by selecting for strains with specific favourable probiotic effects. Thus research lines may be directed at:

- Develop test systems to select promising strategies that positively influence the gut's resistance to infection.
- Determine intestinal effects of various probiotics and prebiotics.
- In particular, examine and optimize the use of microbial populations (starter cultures, probiotics) as means to establish a healthy and stable microbial population in the gut.

Feeding strategies and udder health in dairy cows

Diet also has effects on the expression of immune-related genes in the udder, and may thus modulate immune competence of udder. The dry period is an important moment for dairy cattle, that allows the mammary gland to recover and prepare for the next lactation. It is also a period with a high risk for new intramammary infections and therefore preventive antibiotic therapy is routinely used on a large scale (Bradley and Green 2004). Internationally concern has risen for this overuse of antibiotics.

New intramammary infections during the dry period are often associated with delayed teat closure and leakage of milk (Waage et al. 2001, Dingwell et al. 2004). Low milk production on the day before drying-off significantly improves the closure of teat ends and therefore reduces new intramammary infections (Odensten et al. 2007, Schukken et al. 1993). Furthermore, several natural protective factors, such as phagocytic cells, immunoglobulins and lactoferrin, are found in higher concentrations when cows produce less milk. They serve to protect the udder against intramammary infections, but are diluted with higher milk production (Bushe and Oliver, 1987).

With the high production level of dairy cattle nowadays, milk production at dry-off may often be high (>12 kg up to even >20 kg). Abrupt cessation of milking rapidly decreases milk secretion, but results in higher rates of intramammary infections when compared to dry-off at low milk production levels (Odensten et al. 2007). Reducing milk production at the end of lactation can result in better circumstances to start the dry period. Production can be reduced through changes in the diet and may be combined with reduction of milking frequency end lactation. When cows are dried-off with lower milk yield and better teat closure, the need for preventive and therapeutic use of antibiotics may reduce. Thus we propose to:

- Develop strategies at the end of the lactation period to reduce intramammary infections and the need to use antibiotics. Provide evidence of their effectiveness.
- Understand the mechanisms by which nutrition and feed ingredients affects the udder's resistance to infection.

5. Better diagnostics

Today's livestock production is characterized by tight economical margins and producers need all technological tools to remain competitive. So far antimicrobial use remains a cost effective method to maintain production standards. However, when applied, antimicrobial treatment is often started "blindly", i.e. without specific diagnosis of the etiological agents, their pathogenic properties, and/or their sensitivity to antimicrobials.

Based on clinical symptoms and history of the disease status of the farm, antimicrobial treatment is usually directed at the most probable cause. Confirmation by laboratory tests is not awaited as the inherent delay is regarded unacceptable, because it leads to increased economic losses or even mortality. Instead, the effect of the treatment is often used as a diagnostic tool. Apart from the delay in treatment, it is also argued that the extra costs of laboratory diagnosis are not recovered by less expensive treatment as broad spectrum antimicrobials are widely used.

The use of diagnostic tests can help to diminish antimicrobial resistance in animals by reduction, refinement, and/or replacement of the use of antimicrobials. A major goal is to achieve better treatment protocols based upon proper diagnosis leading to, for instance, the use of small-spectrum antimicrobials or antimicrobials that interfere less with the natural microflora of the host. We expect that parenteral treatments, bypassing the gut with its huge content of bacteria, will result in fewer emergence of resistant bacteria, even though some antimicrobials might reach the intestinal flora via transporter mechanisms. Likewise when diagnostic tests show that disease is virus-associated, the use of antibiotics can be precluded.

Specific and cheaper diagnostics may also assist and optimize the effectiveness of vaccination programmes and other control strategies, leading to a reduction or replacement of antimicrobial use. This may be further facilitated by the availability of diagnostic tools to assess the animal's disease resistance or herd health status.

Thus, in order to achieve reduction, refinement and replacement of antimicrobials diagnostic tools need to be optimized and properly used. Therefore, diagnostic tests need to be fast, robust, cost-efficient and provide results nearby the animal or the veterinarian, i.e. pen-site or vet-site tests. Finally, further refinement and improvement of antibiotic treatments can be achieved by the availability of tests that can detect antimicrobial resistance.

Detection of causative pathogens

Diagnostics for the detection of specific pathogens can be improved in several ways: i.e. by enhancing sensitivity and specificity, by reducing the time-of-analysis, by reducing costs, by allowing earlier detection of disease, and by multi-target diagnostics that not only identify one pathogen, but groups of pathogens, and that simultaneously determine their genotype of phenotype (serotype, toxin production, antibtiotic resistance markers, etc.). The detection and choice of animals to apply the diagnostic procedure to and the interpretation of multiple test results require insight in the epidemiological spread of the pathogens and the pathophysiology of disease.

With respect to antimicrobial usage, it is not expected that increasing the sensitivity and/or specificity of tests will lead to major changes in antimicrobial usage. In veterinary practice, treatment is often started without any use of proper laboratory diagnosis. It is not a lack of sensitivity or specificity that keeps farmers and vets from using diagnostic tests. In order to achieve broader application of diagnostic tests, decreasing the time-of-analysis and costs will likely have a much higher impact on reducing unnecessary antibiotic use provided that such tests are robust and easy to use.

Another means of improving the use of antibiotics is by aiming at an early diagnosis of disease, that is before clinical symptoms become visible. In this way treatment, or preferably prevention, may be started in an earlier stage, which enables the farmer to recover the extra costs of testing and may allow a more effective and sparsely use of antibiotics.

There are several ways to reduce the time-of-analysis. Detecting the causative pathogen can be done by culture methods, which are time-consuming and labour-intensive, or by molecular PCR-based methods. The latter ones can be fast and specific. However, in most samples direct detection by PCR is not yet possible. Several methods are currently being developed combining short culture times (pre-enrichment) followed by molecular (or immunological or biochemical) detection, already reducing the time-of-analysis drastically. Food pathogens such as *Listeria monocytogenes* can be identified in food matrices in less than 27 hours when combining pre-enrichment with real-time PCR compared to ~5 days with conventional culturing (MicroSeq *Listeria Monocytogenes* Detection Kit of Applied Biosystems). The future is to analyse samples without any pre-enrichment step. This requires that protocols must be developed aimed at optimizing the pre-treating of samples.

A different approach is to save time in transporting samples and acquiring results by performing the test on-site at the farm (or slaughterhouse, veterinary practice) where the sample is taken. This is also called pen-site or vet-site. The molecular tools are currently not yet very suitable for on-site use, because they still require well trained personnel. However, they could be feasible for vet-site usage. For on-site or vet-site tests serological assays are well suited. The most convenient on-site tests are lateral flow tests. They are easy to use, cheap and fast. Lateral flow (like the well known “pregnancy tests”) is already widely used for food samples for the detection of zoonotic bacteria, such as *E. coli* O157, *Listeria monocytogenes* and *Campylobacter*. Unfortunately only a few tests for veterinary pathogens have been developed. One example is a lateral flow assay for the detection of antibodies against *Brucella abortus* (Abdoel 2008).

In veterinary practices and slaughterhouses also more sophisticated technologies can be used, as long as they are easy to use, fast and cost-effective. The *Salmonella* and *Trichinella* antibody detection assays by surface plasmon resonance in pig serum are good examples of tests that easily can be performed at the slaughter house (Biacore Herdsense Swine *Salmonella* and Biacore Herdsense Swine *Trichinella*).

A major drawback of present day technology is that currently for every pathogen separate tests need to be used. It is to be expected that multiplex testing will lead to a decrease of costs and may lead to better knowledge of a herd’s disease status as a broader spectrum of pathogens is tested in either individual animals or on herd level, thus enabling the use of a better optimized treatment. Multiplex analyses may thus be used in herd heath monitoring programs, which may help veterinarians and farmers to set up and evaluate systematic treatment strategies employing control measures and vaccinations. Examples are screening tests for a number of animal pathogens, for instance a diagnostic platform for rapid detection and characterization of respiratory or intestinal pathogens.
Mulitplex testing may also reduce time-to-analysis. In answer to the ever-increasing need in biomolecular research and clinical diagnostics to carry out many assays simultaneously in one tube, many technologies have arisen in the past years or are under development.

Examples of popular multiplex technologies are Luminex technology and several micro-array platforms. These platforms can be used for protein-assays, as well as DNA/RNA assays. Multiplex assays can be developed for syndromes, such as respiratory or gastro-intestinal problems. For example, for humans multiplex assays are available for the detection of viral and bacterial respiratory pathogens (xTAG Respiratory Viral Panel of Luminex) (Benson 2007). Comparable assays should developed for the veterinary field.

Finally, while usually diagnosis is directed at the presence or absence of a pathogen, specific characteristics, such as virulence markers or antigenic subtypes, may provide essential information to optimize intervention and epidemiological analyses. In conclusion, major activities will be:

- To develop and evaluate rapid, robust, and cheap diagnostic (multiplex) tests that are able to guide therapy and prevention under field conditions, thus refining, reducing, or replacing the use of antibiotics.
- To include rapid and/or multiple tests for virulence and antigenic (serotype) markers that may be relevant for installation of therapy, prevention, or surveillance purposes.

Detection of antimicrobial resistance markers of pathogens

To detect antimicrobial resistance, the standard techniques of MIC (minimal inhibitory concentrations)-measurements are labour intensive and time consuming. Automation of tests for phenotypic characterization of resistance therefore constitute an important improvement. During recent years increased knowledge has become available on the genetic mechanisms of resistance (i.e. the concerned resistance genes), which enable molecular techniques to be developed. Recent advances for use in academic laboratories include pyrosequencing, denaturing high performance liquid chromatography (HPLC), and nucleic acid analysis by mass spectrometry. None of these technologies is yet suitable for routine diagnostics. These high-throughput technologies allow rapid detection of known single nucleotide polymorphisms (SNPs), including those associated with antibiotic resistance phenotypes, and can also be used for novel SNP discovery (Woodford and Sjundsford 2005).

The challenge is to translate these exciting developments into simplified assays specifically for routine diagnostic purposes. Already on the market are various small micro-arrays for the detection of antibiotic resistance genes. However, it has to be kept in mind that such molecular tools can only detect known antibiotic genes. Unknown resistance genes will be missed. The coming years many new genes and mechanisms will be discovered, which possibly need to be included in diagnostic tools. Also, these tests will only detect the presence of a gene, not its functional properties. However, taken the drawbacks into account, there seems to be great potential for new, fast and specific molecular tools for the detection of antibiotic resistance genes. A particular application for such diagnostic tools could be to help farmers and veterinarians to set up farm-specific treatment protocols. It has been agreed upon by product boards to incorporate such protocols in so called “health plans” set up by farmers and their vets, and updated on regular intervals (Convenant 2008).

Concluding, tests for the detection of antimicrobial resistance may be used in surveillance of resistance of bacteria in animal populations. Importantly, also in practical settings, it enables veterinarians to use more specific and effective treatments. It is important to make this knowledge practically applicable by:

- Developing rapid tests for antimicrobial resistance markers that are useful for therapeutic and surveillance purposes.

Utilizing host parameters for evaluation of herd health

Perhaps the majority of antimicrobials used in animal husbandry is applied to multifactorial diseases. It is even estimated that a major part of the antimicrobial usage is for prevention of such diseases, rather than for therapeutic reasons. In addition to the presence of pathogenic microorganisms, other aspects may play a causative or contributing role in such diseases, such as environmental or nutritional factors.

Because farmers may be uncertain about the presence of certain infections and the health of a group of animals, antimicrobials are often used "just to be on the safe side". It is expected that diagnostic tests that give better insight in the presence or absence of specific micro-organisms together with tests that can assess the animal's health will lead to a strong reduction in "preventive" use of antimicrobials. Especially when these can be coupled to factors in herd management, nutrition, or specific animal diseases.

A new approach might be the development of diagnostic tests directed at the genomic or proteomic response of the host to infection, which may allow to distinguish diseases with a bacterial, viral or non-infectious aetiology, for instance by detection of markers for different immune responses or molecular pathways. Using such diagnostic tests may also help in restricting the use of antimicrobials, i.e. that they will be applied only when relevant.

For the analysis of complex multifactorial diseases, host factors may be investigated in addition to information on the presence of pathogens. Such analysis might need a combination of weighted parameters, most of which are quantitative in nature. Suitable biomarkers have to be identified, combined in an assay, and validated for its predictive and practical use. Biomarkers may provide information on response to infection, immune status, or on the functionality of certain organs, for instance the intestines or respiratory tract. Both at the AHS and at the CVI initiatives have been started to develop diagnostic tests based on blood parameters, such as acute phase proteins, white blood cell markers and liver enzymes.

An alternative approach is the use of diagnostic tools to assess physiological parameters including animal behaviour. For instance the application of wireless sensors to gather data from biological variables (body temperature, heart rate, exhalation gasses), or behaviour (body position, movements, feed intake) by continuous monitoring could be valuable for early warning, as well as to establish the effect of control measures. Such systems are also being developed for the continuous monitoring of elderly people, in which data from biological variables (heart rate, accelerometers, body temperature and galvanic skin response) and everyday habits (body position, movements) will be transmitted to a central monitoring centre. Such systems can also be a valuable tool for the monitoring of herd health.

While these developments are in an early phase of development and may sound futuristic, we acknowledge that there is a need for tests that warn farmers and veterinarians in an early stage, for example in the incubation period of disease, that "something may be wrong". Such an early warning signal may point the way to further (multiplex) pathogen-specific diagnostics, as well as to early and effective interventions before clinical disease and economic harm occur. It may lead to guided and specific treatment of only the sick animals, thus preventing the use of unnecessary antibiotic treatments of all animals present in the herd, or it may lead the way to improved preventive measures. Evidently, such tests need to be cost-efficient. Therefore research will be directed at:

- Development and validation of diagnostic tests of host parameters that may be useful in the early detection of health disorders.
- Definition of their added value in disease prevention and control.

6. Better vaccines and vaccinations

Many good to excellent veterinary vaccines are directed against notifiable diseases, such as foot-and-mouth disease, classical swine fever, etc. One could argue that vaccine development for endemic diseases has somewhat lagged behind in comparison to the major, often viral, notifiable diseases.

Good vaccines may be supportive in eradication strategies and strategies to lower the impact of endemic infections. Moreover, while the "easy" vaccines have been developed, the "difficult" vaccines remain to be developed, both directed against bacterial and viral diseases. Limitations in the development and use of vaccines may be inefficient induction of immunity, in particular at mucosal sites and in maternally immune animals, serotype replacement, the emergence of vaccine-escape mutants, or immune escape mechanisms of the causative pathogens.

The contribution of vaccines to reducing the problem of antibiotic resistance has been shown for example in human medicine. A vaccine directed against *S. pneumoniae* included a serotype that is resistant to fluoroquinolones, and reduced the occurrence of fluoroquinolone-resistant strains (ASM 2009).

Some animal bacterial diseases for which no vaccines are available include necrotic enteritis and *Ornithobacterium rhinotracheale* infections in poultry, and swine dysentery, porcine exudative epidermitis, and porcine edema disease/post weaning diarrhoea (Segers 2009). In addition, no good or suboptimal vaccines are available for certain viral diseases, for which nonetheless substantial amounts of antibiotics may be used to combat secondary bacterial infections, including influenza virus and PRRSV in swine, and BVDV and BRSV in calves.

Although vaccines are available for a number of diseases, they are often not used effectively. We have the opinion that further optimization of vaccine effectiveness using already existing vaccines may be achieved by using better vaccination protocols in a disciplined manner, implying that vaccines are not used (as often nowadays) on an ad hoc, reactive, or therapeutic basis, and thus too late to induce effective protection. For optimal protection, vaccines should be incorporated in a well-designed pro-active prevention program of proven effectiveness.

In developing and evaluating future vaccines it is clearly important to state explicitly the purpose of vaccination, being either 1. to soften or prevent the burden of disease by lowering chances on infection and the expression of clinical signs and economic losses, or 2. to help eradicate the infection by preventing infections and completely stopping the transmission of the pathogen. Note for example that vaccines directed against PRRSV, *Lawsonia intracellularis, Actinobacillus pleuropneumoniae* and *Mycoplasma hyopneumoniae* are available, but these vaccines are currently insufficient to interrupt animal-to-animal transmission of these pathogens, and hence to eradicate these pathogens from swine herds without additional measures.

Major steps to promote advances in this field include identification of diseases for which vaccination may reduce antibiotic use, the rapid identification of protective bacterial antigens, the identification of strategies that pathogens exploit to circumvent mechanisms of natural and acquired immunity, the identification of mechanisms of vaccine-induced protection, identification of strategies directed at particular bacterial functions (e.g. transformation, transduction), optimization of delivery techniques, and optimization of effectiveness evaluation.

New delivery techniques should be based on thorough knowledge of processes, such as antigen presentation, the interplay between the natural and specific arms of the immune system, the induction of protective immune mechanisms, and on the ways pathogens interfere with these processes. Recent insight from genomics research has for example provided insight into molecular pathways that may be involved in the interplay between the natural immune system and the induction of specific immunity, such as the Toll-like receptor signalling pathways. This knowledge should now find its application in vaccine development.

In this work package it is proposed to develop new vaccines directed against diseases that are responsible for a large amount of antibiotic use, that may be candidate for eradication, and for which effective vaccine development is considered feasible. As vaccine development trajectories are long and expensive, we selected diseases and disease complexes that account for much antibiotic use, including the porcine and bovine respiratory disease complexes, both caused by a number of different pathogens (some of which may be unknown or which may have an unknown contribution to the disease), and post weaning diarrhoea/oedema disease of pigs caused enterotoxic *E. coli*.

A vaccine that is protective against multiple serotypes of against *S. suis* is also highly desired. *S. suis* infections in piglets are an important problem in pig industry worldwide. Most herds are infected and a high percentage of the infected herds has acute *S. suis* problems. Considerable numbers of piglets die as the result of a *S. suis* infection. Costs associated with *S. suis* infections in pig industry are estimated to be around 200 million euro/year worldwide. These costs include those associated with the loss of piglets, health care, veterinary practices and the use of antibiotics. Effective vaccines to prevent infections caused by different serotypes are not available. Consequently, antibiotics are very frequently used to treat *S. suis* infections (prophylactically as well as therapeutically). The costs associated with the use of antibiotics (specifically directed against *S. suis* infections) are estimated to be 25-50 million euro/year. High levels of resistance to tetracycline as well as to erythromycin are observed in *S. suis* isolates from piglets as well as from humans. Vaccination of pigs may have implications for human health in areas where human *S. suis* infection is common, for example in China, Vietnam and Thailand. As human infection is considered a zoonosis, reducing disease in pigs may reduce transmission and hence infection in humans.

As a multi-serotype *S. suis* vaccine is currently lacking, herd-specific auto-vaccines are being used. This might be an interesting option, but their effectiveness may be improved by optimized expression of protective antigens, better adjuvants, and they have never been properly evaluated for effectiveness. The same difficulties hold true for *E. coli* autovaccines in poultry.

Research in this work package should in particular be directed on knowledge of the factors that may limit effective vaccine development, and on the evaluation of their effectiveness under field circumstances. In addition to the actual development of vaccines, we will thus pay much attention to their effective use under field circumstances. This entails research on combination-vaccines, delivery routes and mechanisms, and effectiveness against currently circulating herd-specific field strains. We consider the following activities important to let vaccination fully support a policy aimed at reducing antibiotic use.

Optimizing the use and cost-effectiveness of currently available vaccines
A recent questionnaire survey among veterinarians indicated that vaccinations on pig farms were typically initiated because of health problems at the farm (de Groot et al 2004). Vaccine use likely depends on factors such as farm management, whether farmers were allowed to vaccinate, the nature of financial compensation for the veterinarian, and other practical considerations (see for example Holyoake et al 2009). Altogether veterinarians appear restricted in their capacity to disseminate their experience and knowledge due to the competing needs of the farming network (animals, farmers, retailers). Perhaps the most striking feature of this survey is that vaccines are not used properly and for the purpose for which they should be used, i.e. they are often used in response to disease, in stead of well **before** the possible occurrence of disease in order to **prevent** it. Without doubt such a

vaccination practice will only incompletely prevent disease and thus not optimally diminish the use of antibiotics.

Hence many farmers do not use vaccinations as a strategic part of their management guided by a thorough risk analysis, including a thorough diagnosis of strains currently circulating on, or possibly invading their farm, and insight into the cost effectiveness of applied vaccines. In addition, the use of vaccines may be optimized if more combination vaccines would be available, for example directed against PRRSV, influenza virus, porcine circovirus-2, and *Mycoplasma hyopneumoniae*. To optimize the rational use of currently available vaccines, research may be directed at the following questions and goals:

- Design optimal preventive vaccination programs for swine, poultry, and calves under field conditions. Therefore develop tools that allow farmers and veterinarians to design and optimize strategic vaccination plans that are pro-active, (cost-)effective, based on a sound risk analysis, and aimed at minimizing disease and antibiotic use.
- Develop tools to optimally evaluate (cost-)effectiveness of vaccination strategies under field circumstances.
- What are limitations in combining vaccine antigens and how can these be circumvented?
- Identify impediments, whether technical or socio-economic, to implement optimal vaccination strategies.

Improving generic vaccine knowledge and technology

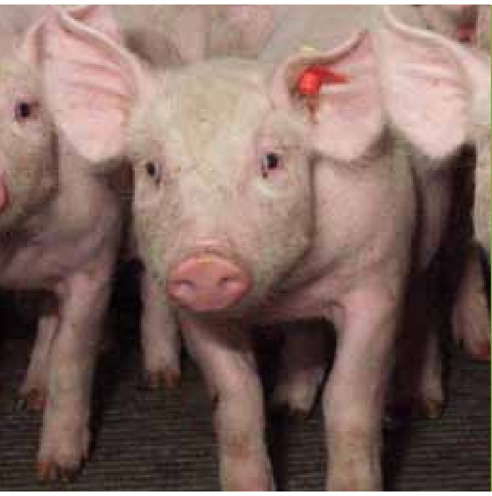
In vaccine development knowledge of protective antigens of specific pathogens is needed. In addition generic knowledge of the induction of protective immune responses is needed that can be applied to specific vaccines.

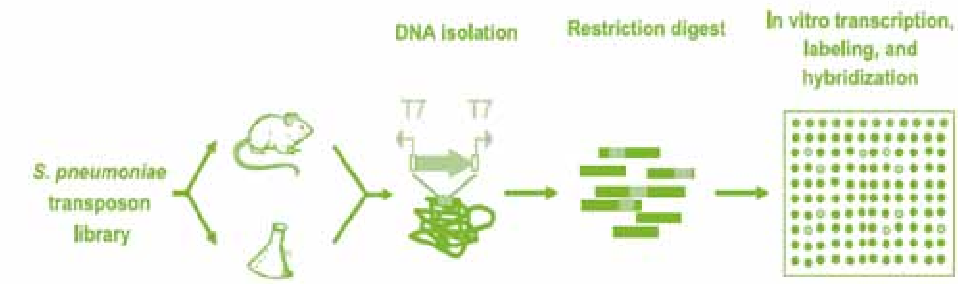
Recently knowledge of the interaction between the natural and acquired arms of the immune system has expanded, knowledge that should now be translated in optimal adjuvants. This knowledge is required since new vaccines are likely to contain less crude extracts of microbial cultures with inherent immunogenic activity in comparison with old vaccines (Heldens et al 2008). In addition, major advances are needed to induce immunity at mucosal sites to non-invading or non-replicating antigens, to circumvent immune-escape mechanisms, etc.

We propose to further enhance our knowledge of vaccine adjuvants that may specifically influence: 1. delivery of antigens, in particular at mucosae, 2. receptors in the natural immune system, and 3. the quality of the immune response (Kapsenberg et al 1999).

An interesting development in this field exploits the use of a defective viral vector that experiences only a single round of virus replication after parenteral application, but effectively enhances the response to co-delivered antigens at mucosal surfaces through a strong induction of type I interferon signalling (Thompsen et al 2006, 2008). Thus this vector has intrinsic systemic and mucosal adjuvant activity. Similar approaches should be explored for livestock animals. Indeed the availability of a mucosal adjuvant that allows to induce immunity by spray vaccination in poultry would reflect a tremendous progress.

Here we further propose to develop molecular techniques aimed at rapidly identifying protective antigens. In particular the use of high-throughput, genome-wide approaches will allow the identification of conditionally essential genes of bacterial pathogens that may be new candidates for vaccines. For example, a high-throughput, genome-wide technique, genomic array foot printing, was developed for *S. pneumoniae*, which allows the identification of genes essential for *S. pneumoniae* at various stages during infection. The principle of genomic array foot printing is shown schematically below.





Schematic representation of the GAF procedure. A large *S. pneumoniae* transposon library is grown under nonselective and selective conditions. Subsequently, chromosomal DNA containing transposons (gray rectangle) with outward-facing T7 RNA polymerase promoters (arrow with T7) is isolated from each population. The DNA is digested, and the DNA adjacent to the transposon insertion site is amplified using in vitro transcription with T7 RNA polymerase. The RNA is used in standard microarray procedures. Hybridization of these probes to a microarray will reveal which genes were disrupted in the mutants that disappeared during selection: those spots to which only material derived from the nonselective condition will hybridize (gray spots).

A transposon library is constructed making use of a transposon flanked by outward-facing T7 polymerase promoters. Genomic array footprinting detects the transposon insertion sites in the library by amplifying (transcription with T7 RNA polymerase) and labeling the chromosomal DNA adjacent to the transposon and subsequent hybridisation of these probes to a microarray. Identification of transposon insertion sites that have disappeared from the library due to selection is achieved by differential hybridisation of probes generated from the library grown under two different conditions to an array. Therefore, genomic array footprinting will enable the identification of genes essential at various stages during infection. This technique may be combined with genome-wide approaches to assess the immunogenicity of gene-products. Together such approaches will lead the way to candidate antigens which can be used for the development of novel vaccines.

Specific goals and research questions are:

- How can we rapidly identify immune mechanisms responsible for protective immunity?
- How can induction of protective immunity be optimized, especially at mucosal sites and directed at inactivated vaccine antigens? In particular, how can pathways of antigen recognition and presentation be optimally activated by new types of adjuvants or immune potentiators? How can we selectively stimulate essential immune pathways with a minimum of adverse reactions?
- How can immune suppressive and immune escape mechanisms (including low immunogenicity) of pathogens be circumvented?
- How can we circumvent serotype-specific immunity? How can we prevent adaptation or escape of bacteria from vaccine-induced immunity? How can we optimally adapt vaccines to changing pathogens? What should we know about pathogen evolution in vaccine development?
- How can we optimize and use genomic tools to rapidly identify and select candidate vaccine antigens?

Optimizing vaccine use in relation to genetic background

It is evident that polymorphic host genes determine the response and hence efficacy of vaccination (Kimman et al 2007). However this knowledge has not been translated in vaccine development or vaccine used tailored to a host genetic factors, such as different breeds, lines, etc. The highly polymorphic leukocyte antigen system, which is involved in antigen presentation, has been researched most in this aspect, and clearly affects the response to vaccination. Other, but less polymorphic pathways involved are the Toll-like receptor pathway, which is involved in antigen recognition and stimulation of the immune system, and the cytokine immunoregulatory network.

Clearly both antibody and cell-mediated responses are not only affected by loci within, but also strongly by loci outside the human leukocyte antigen system, such as recognition and signalling modules of immune system. Because most genes that are important in influencing immune responses to vaccination are still unknown, more work is required. A better understanding of the host factors that determine an effective response to vaccination may lead to the identification of specific genes and pathways as targets for the development of novel, more uniformly effective vaccines, and to application of vaccines tailored to specific breed or individuals. Concrete research questions are:

- How large is the influence of genetic variation on the magnitude and efficacy of vaccine-induced immune responses?
- Which polymorphic host genes and genetic pathways determine the effectiveness of vaccine-induced immune responses?
- How can vaccination programs be optimized to specific breeds or genetic backgrounds? What are optimal gene-vaccine combinations?

Developing vaccines against diseases that necessitate large antibiotic use

In order to develop vaccines against specific diseases a tailored approach is needed taking protective antigens, protective mechanisms, and aspects of pathogenesis into account. These are all aspects that may be necessary to study in the development of a specific vaccine. Now with rapid whole genome sequencing and expression techniques available, the challenge is to exploit these techniques in rapid identification of candidate vaccine antigens, virulence genes, and subsequent vaccine development.

In selecting pathogens for vaccine development we have in particularly selected pathogens for which antibiotics are likely used in large amounts. (Note that precise data on antibiotic use for specific diagnoses is lacking at the moment.) Hence we did not primarily select disease problems that may cause zoonotic infections in humans (*Salmonella, E. coli, Campylobacter, Staphylococcus aureus*), but that do not cause much disease and accompanying antibiotic use in animals themselves. (Note however that antibiotics may under strict regulations be used for treatment of specific Salmonella infections in breeding poultry). Nonetheless vaccines against these diseases might be part of eradication and control programs aimed at reducing zoonotic transmission, and hence the use of antibiotics in humans.

The potential of the strategy to use vaccines in attempts to reduce antibiotics is documented for example by the reduction in use of antibiotics after the introduction of vaccinations of salmon against furunculosis, caused by Aeromonas salmonicida) and cold-water vibriosis in Norway. A reduction of the use of antibiotics of 98 % was subsequently achieved (Segers 2009, Markestad and Grave 1997, WHO 2006). A Danish study showed that vaccination of pigs against *Lawsonia intracellularis* reduced the consumption of oxytetracycline to treat PIA by 79%, also with a significantly lower number of pigs being treated. Vaccination further resulted in a highly significant improvement of average daily weight gain and carcass weight, as well as a shortened fattening period (Bak and Rathkjen 2009).

Finally, vaccination can also be designed to fight resistant bacteria, for example MRSA. Development or improvement of vaccines against the following diseases and disease complexes may drastically reduce antibiotic consumption and antibiotic resistance in animal husbandry.

Develop or improve vaccines against:

- PRRSV. Although a viral disease, PRRSV causes reproductive disorders in sows and enhances sensitivity of fattening pigs to several respiratory bacterial pathogens. It is therefore responsible for a large antibiotic use.
- *S. suis* in pigs.
- Porcine post weaning diarrhoea/oedema disease. Although optimization of weaning may reduce the incidence of this disease complex, a vaccine may further substantially reduce antibiotic use of pigs during this period.
- Swine dysentery, caused by *Brachyspira hyodysenteriae*.
- Necrotic enteritis in poultry, caused by *Clostridium perfringens*.
- *Ornithobacterium rhinotracheale* in poultry.
- *Avibacterium paragallinarum* in poultry.
- Colibacillosis in poultry.
- Respiratory disease complex in calves, and respiratory disease complex in pigs, both caused by a multitude of pathogens. Combination vaccines optimized to local and current circumstances may be of great benefit.
- MRSA.

7. Alternatives

Evidently most research lines in this White Paper are directed on disease prevention and on developing a "pathogen-free" and "drug-free" animal production, rather than on finding something else for the currently used antibiotics. Nonetheless we should not completely overlook the possibility of finding news drugs or treatment methods provided that they do not carry the risks of the current antibiotics, for example that their use does not obscure inherent management disorders, does not lead to the emergence of resistance against the new alternative drug, does not lead to long-term negative ecological consequences, and does not interfere with human therapy and public health. Hence that their use can be responsible and sustainable.

New alternatives for classical antibiotics?

A way of preventing the further emergence of resistance would be to introduce novel antibacterial medicines with modes of action distinct from conventional antibiotics. Research in this direction is partly financed in the framework of the ALTANT program organized by ImmunoValley.

A range of approaches has been suggested to develop "new" antibiotics or alternatives, including bacteriophages, lytic enzymes derived from them, such as bacteriolysins and colicins, antibacterial peptides, lantibiotics, etheric oils, organic acids, plant herbs and other pytopharmaceuticals, olive oil, bacterial apoptosis inducers, agents interfering with bacterial virulence factors, modulators of bacterial transcription, adhesion-inhibitors, specific antibodies, etc, etc (Bast 2009, Engelberg-Kulka et al 2006, Fink-Gremmels 2009, Fischetti 2005, Borysowsky et al 2006, EFSA 2005, Keyser et al 2008, Wiley and Van der Donk 2007). So far these approaches have had limited success in actually developing and marketing new products.

As example, interest in plants, plant extracts and derived phytochemicals as components that can enhance health and productivity has increased during the last decade (Griggs and Jacob, 2005, Rickard, 2004, Wallace et al., 2009). Botanicals may not only be considered as replacements for classical antimicrobial growth promoters, but they may also have other useful properties. Herbs, spices and their extracts can stimulate feed intake and endogenous secretions (Wenk, 2003). Many botanicals have antioxidant activities that can improve the oxidative stability of poultry meat and eggs, increasing their shelf life. They may also stimulate immunity directly, improving the animals' resistance to disease. They also have the potential to modify cholesterol metabolism, potentially leading to a healthier product for human consumption (Durape, 2007). However, many plants and phytochemicals have adverse effects (Wallace et al., 2009, Acamovic and Brooker, 2005). Recent concerns about the use of synthetic compounds to enhance performance has now stimulated interest in the useful effects of phytochemicals in the diets of farmed animals.

Difficulties that alternatives may meet are resistance to the alternative (bacteriophages, antibacterial peptides), toxicity, other side effects (garlic), a very limited antibacterial spectrum (for example a specific bacterial toxin), lack of scientific evidence of efficacy and mechanism of action, high costs, limited market potential. And, of course, they are mostly therapeutic and not preventive tools. As a result, there are currently almost no new candidate antibiotics in the pipeline of industry to replace the existing classical antibiotics.

A limiting factor in developing new antibiotics could be the tools that have been used in the selection of alternatives, i.e. most researchers have so far only used classical growth inhibition tests. Alternative methods for selection may, for example, be directed on microbial strategies to circumvent immunity, on strategies interfering with bacterial communication (quorum sensing), bacterial toxicity, programmed cell death, or bacterial survival strategies (Moy et al 2009). Recent studies of bacterial virulence factors and toxins have for example resulted in increased understanding of the way in which pathogenic bacteria manipulate host cellular processes. Such knowledge may now be used to develop novel antibacterial medicines that disarm pathogenic bacteria.

We propose that research may be directed on a few promising approaches that may have a broad, generic mode of action, and, in particular, on the hurdles that may prevent their widespread use, including potential evolutionary forces resulting in newly emerging resistance to the alternative. A thorough risk-benefit analysis should therefore accompany research on alternatives. Important research activities are:

- To identify novel molecular pathways as new targets for intervention in bacterial infections.
- To develop new (high throughput) selection systems (in vivo, in vitro) for selecting new drugs without the “current” disadvantages.
- To formulate requirements for alternative drugs based on a risk assessment approach.
- To develop test models in which the effects of novel, alternative treatments on microbiological, clinical and economic outcome parameters following infectious challenge can be established and validated.

Exploring bacteriophages

In particular we consider it promising if alternatives are directed towards specific use in the animal population, and do not compete with application in humans. Such an approach will prevent transmission of possible resistance mechanisms to the human population. One of these approaches may be the use of bacteriophages, the natural viruses of bacteria that selectively kill bacteria (Adhya and Merril 2006, Vaneechoute et al 2009).

Hurdles in the widespread application of bacteriohages are the emergence of resistance to bacteriophages (in fact it is an inherent natural characteristic of their evolving biology that should be exploited optimally by selecting new phages with new therapeutic possibilites), the very narrow spectrum (or high specificity) of bacteriophages, suboptimal distribution in the body, antibody formation upon repeated administrations, and a sometimes incomplete efficacy. Note that there are numerous types of bacteriophages each killing only a few bacterial subtypes.

However, many promising data on the effectiveness of bacteriophages in treating bacterial infections have also been reported, including the treatment of vancomycin-resistant *Enterococcus faecium* and *E. coli* infections of the gut (Fink-Gremmels 2009, Smith et al 1987).

Another example is LISTEX that contains a bacteriophage specifically killing *Listeria monocytogenes*. This is the first phage-containing product that is recognized by the FDA as being safe for application in food (Van der Vlugt and Verbeek 2008).

Some of the disadvantages of bacteriophages may prohibit their use in humans, restrict their use to animals, and hence make their application for animals even more acceptable and therewith promising. Application of bacteriophages may be (even must be) supported by specific diagnostics regarding the sensitivity of the pathogenic bacteria to specific phages, and varying use of different phages, or combinations thereof, i.e. cocktails. In particular we hypothesize that short-term bacteriophage treatment may be helpful in promoting a beneficial shift in enteric bacterial populations.

It is not to be expected that phage therapy will kill 100 % of a bacterial species, but as an adjunctive therapy such a high level of efficiency may not be needed. Hence we propose exploiting knowledge of the vast variation of bacteriophages, their evolving nature, and their natural resistance patterns rather than making resistance prohibitive to their use. The narrow spectrum of bacteriophages may be considered advantageous since there is little risk of cross-resistance. An important advantage of phage therapy is that they are completely safe. Enhancement of their antibacterial efficacy may be achieved by genetic modification, for example by incorporation of genes expressing lytic enzymes, or by modifications that increase their lifespan or optimize their biodistribution.

Bacteriophages, since they are living and flexible organisms, may also be "trained" by natural means for optimized characteristics. Thus,

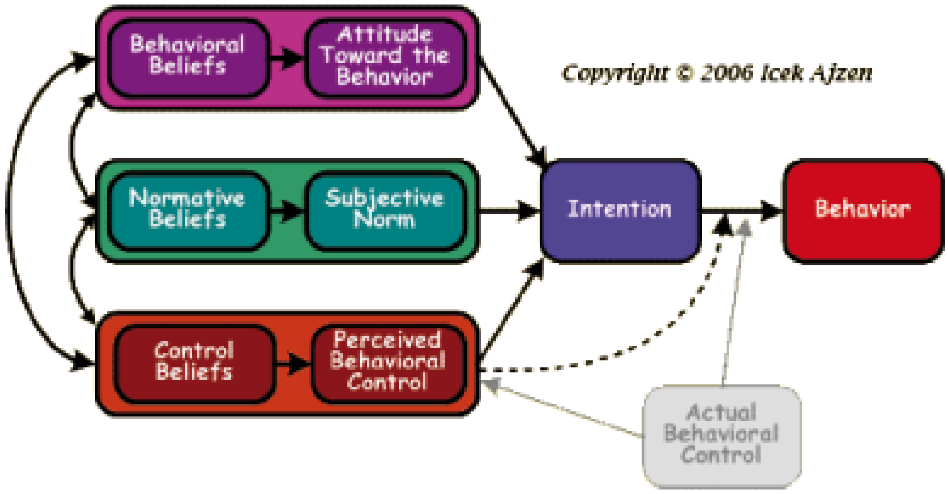
- Explore the possibilities of phage therapy for use in animals. In particular select a few infectious diseases (for example swine dysentery, weaning diarrhoea, S. suis) that may benefit from phage therapy. Take consequences for public health into account.
- Develop application methods (mixes, sequential application of phages, etc) that circumvent phage resistance or minimize its consequences.
- Explore the possibilities of optimizing phage therapy, for example by genetic improvement of phages.

8. Implementing changes

Reducing antibiotic use is a common interest for animal husbandry and public health. The current practice of animal management and antibiotic use, attitudes, laws, regulations, must be changed, and this should be possible thanks to technical improvements. However, as generally, known, it may be very hard to alter behaviour and cultural habits. Implementing the different possible measures needs an integral approach.

To study the potential effect of different measures on the behaviour of the farmer and to help identifying vital areas for further research, a conceptual framework may be helpful. For this purpose we use the Theory of Planned Behaviour (TPB) (see text box), since it is an often used conceptual framework to describe intentional behaviour and behavioural change. (Bergevoet 2005).

The Theory of Planned Behaviour (Ajzen 2006) explained.



Components of the TPB are:

1. Behavioural beliefs link the behavior of interest to expected outcomes. A behavioral belief is the subjective probability that the behavior will produce a given outcome.
2. Attitude toward a behavior is the degree to which performance of the behavior is positively or negatively valued. Specifically, the strength of each belief is weighted by the evaluation of the outcome or attribute, and the products are aggregated.
3. Normative beliefs refer to the perceived behavioral expectations of such important referent individuals or groups as the person's spouse, family, friends, and – depending on the population and behavior studied - teacher, doctor, supervisor, and coworkers. It is assumed that these normative beliefs - in combination with the person's motivation to comply with the different referents – determine the prevailing subjective norm.
4. Subjective norm is the perceived social pressure to engage or not to engage in a behavior.
5. Control beliefs have to do with the perceived presence of factors that may facilitate or impede performance of a behavior.
6. Perceived behavioral control refers to people's perceptions of their ability to perform a given behavior. Intention is an indication of a person's readiness to perform a given behavior, and it is considered to be the immediate antecedent of behavior.
7. Actual behavioral control refers to the extent to which a person has the skills, resources, and other prerequisites needed to perform a given behavior. Successful performance of the behavior depends not only on a favorable intention but also on a sufficient level of behavioral control.
8. Intention is an indication of a person's readiness to perform a given behavior, and it is considered to be the immediate antecedent of behavior.
9. Behavior is the manifest, observable response in a given situation with respect to a given target.

Several measures are suggested (also in this White Paper) that may contribute to change behaviour of farmers to reduce of antibiotics in intensive livestock farming: These items will be discussed by placing them in the TPB.

- **Preventive measures** like vaccines, nutrition and other preventive measures. Having preventive measures available facilitates to change behaviour. Characteristics of successful measures are amongst others, ease to implement, contribution to elimination of the problem, success rate of the measure, and cost effectiveness. These are important factors in enabling actual behavioural control.

- **Study groups/ peer groups /farmers networks** are groups of farmers learning from each others. They are characteristic for Dutch agriculture. Study groups have a role in supporting or changing the normative beliefs of farmers by discussing the subjects related to antibiotic use. They support the actual and perceived behavioural control by learning from each others best practices (Bergevoet and Woerkum 2006, Wielinga et al. 2008). In Denmark good experience in reducing antibiotic use has been obtained using the farmer field school (FFS) concept for farmers' learning, knowledge exchange, and empowerment that has been developed and used in developing countries. A research project focusing on explicit non-antibiotic strategies involved farmers who had actively expressed their interest in phasing out antibiotics from their herds through promotion of animal health (Vaarst et al 2007).
- **Legislation and regulation.** Adequate human health care can be jeopardized by the behaviour of individuals (e.g. farmers using antibiotics). Enabling adequate human health care that includes the ability to treat with antibiotics, is a governmental responsibility. But also the production chain, forced by consumers' perception, considers this increasingly as their responsibility. Legislation and regulation can be used to control an excessive use of antibiotics. Legislation is a tool of the government to prevent unwanted behaviour and enforce favourable behaviour by individual actors. Regulation can originate from the government, but also from private parties (e.g. buyers or chain parties). Both can be used to enforce specific behaviour of individual actors. Many actors intrinsically want to comply with legislation and regulation since they accept and agree with the regulation and/or are not willing to bear the consequences in case they do not comply (willingness to comply and normative beliefs). Implementing legislation and regulation has consequences for the farmers behaviour. It might be perceived and actually affect behavioural control. However, if the regulation is not enforced, the attitude of the farmer and significant peers (normative beliefs) towards this legislation might change and reduce the motivation to comply. Clear objectives may be instrumental.
- **Attention in public and professional media.** Media attention (positive and negative) influences the normative beliefs of farmers. An example may be reports on the consequences of antibiotic use for the farmers' family health. Reports on successful efforts of relevant peers can affect the perceived behavioural control of individual farmers: "If (s)he can manage it, it should be possible for me as well". Reports on unsuccessful efforts have a negative effect on this perceived behavioural control.
- **Economic incentives** have an effect on the actual behavioural control of farmers. If implementing changes results in additional efforts and costs, economic incentives can help to remove barriers.
- **Education and training and identification and communication of best practices** influence the actual and perceived behavioural control. These efforts affect the capability to successfully implement the change. Data of antibiotic use of farmers and, or veterinary practices (see WP 9) may be used for benchmarking, and formulating requirements of antibiotic use and prescription behaviour.

The challenge of farmers and policy makers is to assess each of these items on its own merits and evaluate the extent to which they contribute to the reduction of antibiotics use (Fig. 9). Needed. is an evaluation of the effect of each item on it self, but also in combination with other items. It is obvious, however, that no single measure by itself is able to change the behaviour of farmers towards a reduction in the use of antibiotics. Some items, for example legislation, might on its own not reduce the use of antibiotics, but may be a vital precondition for other items to be successful.

While it is important to address farmers, we consider it also important to address other stakeholders as well, in particular the meat and milk industries, retail organizations, and consumer organizations. These organizations may in particular be important by demanding a certified and guaranteed antibiotic-free or antibiotic-poor way of producing.

While several approaches may lead to different ways to prevent or to diminish the use of antibiotics, it is important to integrate and fine-tune these approaches into comprehensive plans of work. This requires insight in the relative contribution and synergy of different approaches, as well as their (cost-)effectiveness. Decisions in these matters may benefit from the use of decision-support models.

Furthermore, it is of utmost importance to identify and overcome educational, organizational and economic hurdles in the prudent use of antibiotics or in the implementation of other approaches to prevent their use, such as pathogen-free farming, etc. Therefore suggested changes should at least be cost-effective for farmers and acceptable for society. In addition, it is important that measures are coordinated at international (EU, OIE) level.

Activities in this area will be supportive to initiatives such as described in the Convenant antibioticaresistentie. Wanted activities include to:

- Define which conditions have to be fulfilled to stimulate reduction in the use of antibiotics.
- Get insight into the timing and order of the implementation of the different measures.
- Make an inventory of impediments that should be overcome, for example lack of knowledge among farmers on correct use and long-term consequences of antibiotic use, or economic constraints.
- Examine which positive and negative incentives may be supportive in establishing a cultural change, i.e. in changing attitude and behaviour towards the use of antibiotics. Examples may include benchmarking, higher prices for farmers that produce without antibiotics, taxes on antibiotics, and division of responsibilities regarding diagnosis of disease, prescription of antibiotics, and sale of antibiotics.
- Get insight whether differences between farmers needs different approaches.
- Examine governance and management aspects aimed at reducing antibiotic use both at national and international levels. Take experiences in other countries into account. Take the potential contributions of national and international governing bodies, and professional, retail and consumer organizations into account.
- Therewith define the best governance practices to reduce antibiotic use in animal husbandry.
- Examine how other stakeholders, such as the meat and milk industries, retail organizations, and consumer organizations, may be involved by demanding or promoting a certified and guaranteed antibiotic-free or antibiotic-poor way of producing.
- Optimize knowledge transfer from the scientific domain to the field.
- Explore the possibilities of farmers' innovation networks to act as forerunners and examples, and examine how such networks should be supported so that their results can be optimal and that their experiences can be disseminated quickly to the broader agricultural community.
- Provide evidence of the effectiveness of suggested measures aimed at establishing shifts in attitude and actual behaviour towards the use of antibiotics.

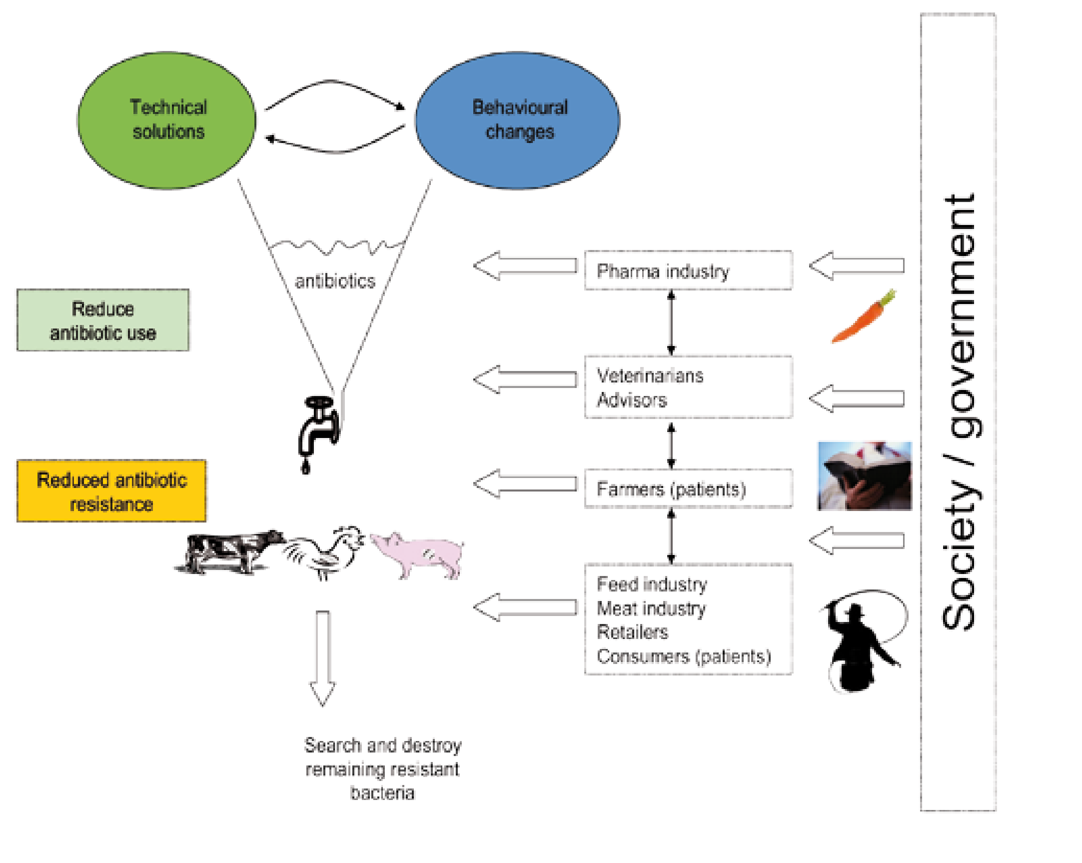


Fig 9. Major actors in the field that each play roles in the challenge to reduce antibiotic use are farmers, backed by their veterinarians and advisors, and the pharma, feed, and meat industries. Additional roles may be executed by retail and consumer organizations. Evidently a major role in promoting behavioural changes in this area is for the government.

9. Registration and monitoring of antibiotic usage and resistance

Our efforts must lead to reduced antibiotic use, diminished resistance and diminished transfer of resistance. To follow these trends and to receive early warning signals of setbacks or new problems, it is of utmost importance to optimize the current monitoring- and surveillance systems.

Monitoring of antimicrobial resistance is a statutory task in all EU-members states and is coordinated by designated National Reference Laboratories. Sampling strategies, microbiological methods for strains identifications and susceptibility tests are prescribed in detail by EFSA. These phenotypical data provide information on the occurrence and trends in resistance for a predetermined panel of antibiotics in The Netherlands and Europe in sentinel organisms (*Salmonella, Campylobacter, E. coli and enterococci).* Other organisms of public health concern are not specified by EFSA and therefore not included unless they are specifically named, as was done for animal-derived MRSA in a specific baseline study. This means that monitoring of resistance on a national level is well organized. However, this type of information is inadequate for epidemiological studies on farms aimed at risk factors for resistance. For such studies resistance needs to be quantified by farm or holding or even pen, depending of the study design.

Currently solely phenotypical methods are used for resistance surveillance as described above. Although the choice of these sentinel organisms is rational, resistance may occur and spread through a variety of other organisms (e.g. anaerobes) not included. Since the public health concern of resistance in animals is not only related to certain resistant organisms (e.g. MRSA), but also to the occurrence and spread of resistance genes (e.g. ESBLs), the current approach for surveillance may miss essential information on dissemination of resistance. An approach in which resistance determinants (genes) are directly detected in matrices like surface water, faeces, waste water plants etc. would be of great importance to study the transmission of these genes within and between certain environments. These novel metagenomic approaches would include PCR on isolates, characterization of (plasmid) DNA, random sampling pyrosequencing, or microarrays.

The use of antibiotics in the animal husbandry is an important determinant for occurrence and increase in resistance in both veterinary and human pathogens (FAO/OIE/WHO, 2003). Interventions in animal production should aim to reduce overuse and misuse of antimicrobials on farms (WHO, 2000). Because in food-producing animals during mass-medication a high proportion of the animals treated and exposed to antibiotics is healthy, although at risk of becoming infected, it should be possible to reduce the necessity for antimicrobials in agriculture, among others through adoption of Good Animal Husbandry and Good Veterinary Practice (FAO/ OIE/WHO, 2004). This is important as it will lead to a reduced risk of antibiotic resistance in the food chain (Aarestrup and Seyfarth, 2000; Barza and Sherwoord, 2002; Catry et al, 2003; Silbergeld et al, 2008; Wegener et al, 1999; WHO, 2003). Transparency in antibiotic usage and insight in the rationale to use antimicrobials in the Netherlands is needed to develop well founded interventions on veterinary antibiotic use and to cultivate a different antibiotic policy. It is therefore of utmost importance to monitor trends in antibiotic use in a quantitative way.

Registration of antibiotic usage in The Netherlands is currently done by sales data of FIDIN. Detailed and quantitative information of usage is expressed as defined daily dosages per average animal by LEI. The FIDIN data allow a very rough estimation of trends in usage, because the sales data cannot be attributed to animal species or types of administration. The LEI system specifies antibiotic usage in numbers of dosages per animal per time period and for each route of administration and each antibiotic class (daily doses/animal year [dd /ay]). Because of the denominator data included in the units of measurement, this type of information adequately describes the exposure of animals to different antibiotics. This type of information can very well be used for epidemiological studies aimed at trends in usage. Both the FIDIN and LEI information are used in the Dutch national reportage on data of animal consumption of antibiotics. In contrast to monitoring of resistance, monitoring of antibiotic consumption is not mandatory in the EU. Hence there is no EFSA protocol for monitoring antibiotic use in Europe. In addition, the licensing system differs by EU-member State.

In addition to the LEI and FIDIN activities, there are currently several activities on antibiotic usage registration which are all related to the Convenant that the animal production sectors have signed with the aim to stimulate the rational use of antibiotics. FIDIN/AUV and KNMvD are developing a database for prescription of all antibiotics by veterinarians (VETCIS). This provides an opportunity to evaluate all prescriptions. Moreover, VETCIS can be used to study exposure in detail (LEI-manner), if it can be linked to database on farms. A drawback is that it is a voluntary system and not all essential information (as listed below) is included. In fact it is developed as a tracking and tracing system.

To study the attribution of certain antibiotic usage practices to resistance a well defined study design is essential in which both antibiotic usage and resistance are quantified in a panel of farms. Both the methods used and the numbers of farms included should aim at the ability to determine statistically significant determinants for high or low levels of resistance. These studies may help to define antibiotic usage strategies with less potency for selection of resistance.

In conclusion, activities in this work package will be directed on:

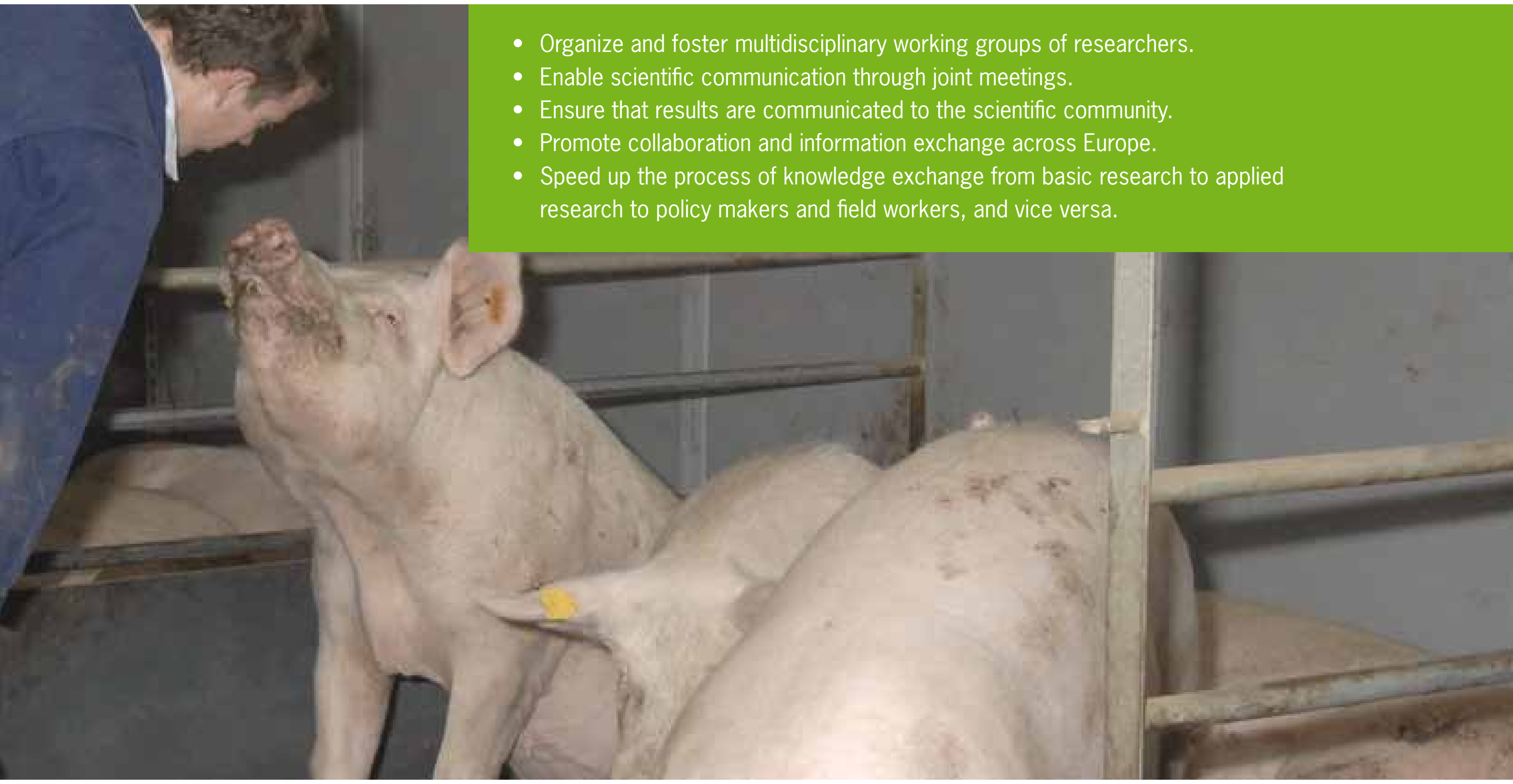
- Developing, optimizing, and harmonizing methods for quantification of resistance in populations (e.g. farm level, national level).
- Optimizing methods for quantification of antibiotic usage in populations.
- Identification of risk factors for antibiotic usage for respectively high and low levels of resistance.
- Relating trends in antibiotic use with trends in antibiotic resistance, including the potential transfer of resistance from animals to humans. Preferably establish causative relationships.
- Optimizing methods of providing animal owners, advisors, veterinarians, and policy makers with feed-back data.
- Developing tools for metagenomic analysis of resistance genes in different matrices enabling a study of transmission routes of resistance in the environment, surface water, food chain, etc.

10. Communication and scientific exchange

The research efforts aimed at reducing antibiotic use in animal husbandry are complex, manifold and multidisciplinary. In fact most of the work should be of a multidisciplinary nature. To promote that the researchers in this field can speak the same language, use the same models and terms of reference, and optimally exchange their results and work plans, it is of utmost importance to specifically address communication and scientific exchange between them. In addition, it is important to ensure that the results of their work will be distributed to relevant stake holders, such as veterinarians, farmers, and policy makers.

Because we may expect that regulations in the field of antibiotic use may be based on EU laws, we consider it important to seek and promote collaboration at the European level, both with researchers and policy makers. We feel that it is an urgent matter that antibiotic use in the animal production sector is reduced, and that research should – as quickly as possible- enable stakeholders to take their responsibility. Hence it is important to:

- Organize and foster multidisciplinary working groups of researchers.
- Enable scientific communication through joint meetings.
- Ensure that results are communicated to the scientific community.
- Promote collaboration and information exchange across Europe.
- Speed up the process of knowledge exchange from basic research to applied research to policy makers and field workers, and vice versa.



Antibiotic resistance in aqua culture

The focus of this White Paper is directed on the main production animals, i.e. swine, bovines, and poultry. However, aqua cultured fish production, in particular of pangasius and shrimp, is a fast growing economic activity, and antibiotics are abundantly used in this sector. Many fish and fish products, either imported from abroad or not, are contaminated with antibiotics. Only few antibiotics are registered for use in cultured fish, and there appears to be a general lack of knowledge on their proper use and effectiveness. However, with the highly controlled and closed systems of commercial aqua culture, a strictly controlled way of pathogen control, using few or no antibiotics, must be possible.

We therefore recommend, following the main lines of this White Paper, to:

- make an inventory of antibiotic use, antibiotic resistance, and transfer of resistance markers in and from the aqua culture sector (i.e. problem definition), and, based upon the results,
- to explore the possibilities of working "pathogen-free" in aqua culture,
- to diminish the impact of unavoidable infections ("disease elimination"), and
- to reduce, refine, and replace the application of antibiotics when their use is unavoidable.

Relationships between work packages

It is evident that the research lines described in the White Paper contribute to different research aims, but are strongly interrelated. In Fig. 10 we have indicated these relationships.

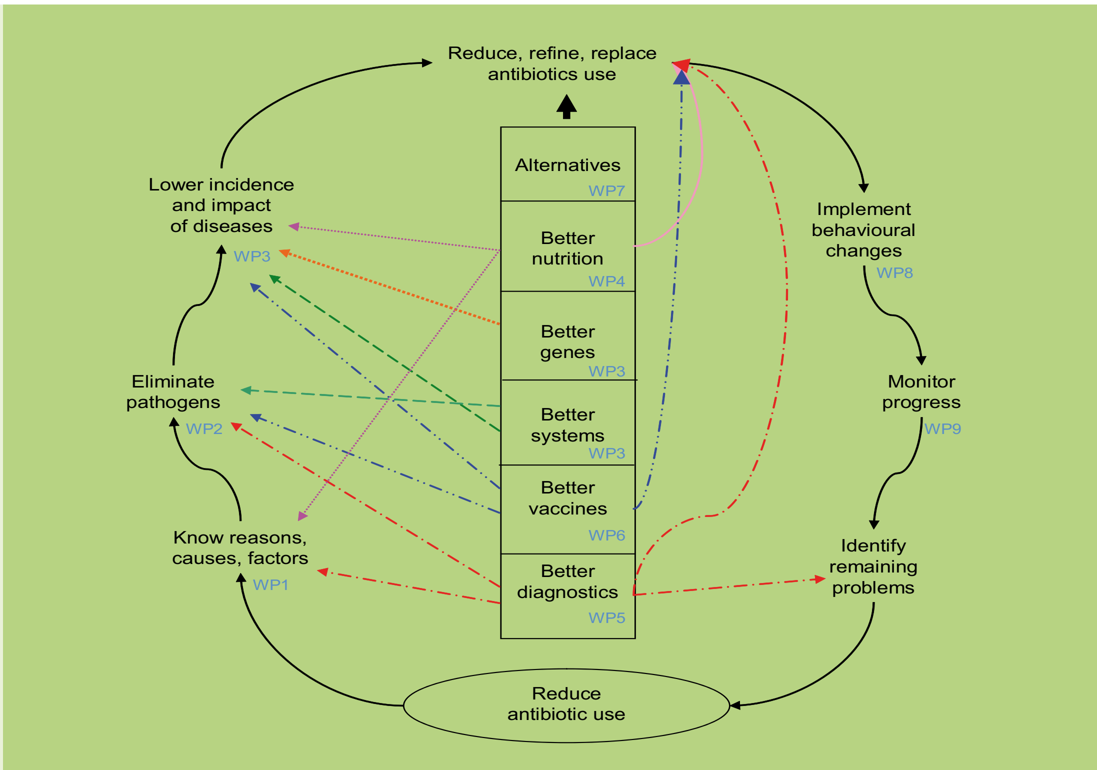


Fig 10. Relationships between work packages and research aims. To reduce antibiotic use, the first aim is to know the reasons, causes, and factors that determine antibiotic use and resistance (WP1). Building on this knowledge, further research aims are directed on elimination of pathogens or the pathogen-free design of animal husbandry (WP2), or, if impossible, on lowering the incidence and impact of infections (WP3). These research aims heavily rely on the activities described in the boxes: the improvement and development of better diagnostics (WP5), better vaccines (WP6), better zootechnic systems and genetic backgrounds (WP3), and better nutrition (WP4). Together with the development of new alternative treatments (WP7), these developments may lead to reduced, refined, or replaced use of antibiotics resulting in diminished emergence and selection of resistance. A separate work package is directed on the requirements for governance, and behavioural and attitudinal changes related to the use of antibiotics (WP8). Surveillance will allow to monitor progress (WP9), which together with diagnostics (WP5) might identify new or remaining problems to be addressed.

Remaining unresolved issues

In doing research enabling an antibiotic-free animal husbandry and in actually implementing such a system, we may expect some as yet unresolved and unforeseen issues and questions, for which we should not close our eyes and which should receive attention in the future. It is for example not yet known which research and which actual changes will yield the most favourable results, and which efforts will be most cost-effective. Relationships between observed parameter changes in the laboratory and antibiotic use in the field will likely not be linear. For example, if animals have a 10 % lower chance of acquiring an infection, what will be the impact of this finding on antibiotic use in the field?
Another unresolved issue, is what the remaining antibiotic resistance will be after adequately banning much of the current antibiotic use. Although we may expect a gradual fading out of resistant bacteria after the disappearance of antibiotics as selective forces, it is not yet clear if this would be general true for all bacterial species and resistance markers, what the rate of such disappearance of resistance would be, what other factors would determine it, and if additional measures should be taken to control remaining resistant bacteria, such as a "search and destroy" policy. Unfortunately, for example, continuing high prevalence of VanA-type vancomycin-resistant enterococci occurred on Norwegian poultry farms three years after avoparcin was banned (Borgen et al 2000). This may indicate a remarkable stability of the VanA resistance determinant in an apparently non-selective environment.

It is also yet unknown what should and could be the wanted or required "real minimum" of antibiotic use. On the one hand we may strive towards a zero-level, thus completely eliminating any evolutionary force on antibacterial resistance, but we cannot yet foresee whether such a zero-level (for all antibiotics and bacterial spp.) is really required to eliminate resistance, or whether a certain level of refined and careful use of antibiotics may be allowed. We can also not yet foresee, but should define it, to what extent intensive animal production can really produce effectively and sustainably without the use of antibiotics, and what the economic constrains thereof would be. Therewith we have the opinion that there should always be room to treat animals for unforeseen, newly emerging, and accidental infections in a careful and responsible manner. Failure to do so would lead to unacceptable animal suffering.

In aiming to diminish the systematic use of antibiotics we should strive to provide sustainable solutions, characterized by lack of negative evolutionary consequences, long-lasting effectiveness, safety, lack of transfer of negative consequences to future generations or other parties, and societal acceptance. This includes that solutions have to be acceptable for farmers and veterinarians, and must allow a profitable animal husbandry.

Farmers have a large responsibility for the health and well being of their animals (and in addition to the consumers of their products), and they bear large economic risks. It is therefore understandable that they use antibiotics to minimize disease risk. Thus, although several lines of research may seem to raise costs (for example through increased costs of vaccinations, diagnostic tests, or more expensive treatments), future innovations in animal husbandry will only be sustainable if they improve the quality of disease prevention and animal husbandry in a long-lasting manner that does not interfere with economic requirements. We may expect that efforts will improve the quality and uniformity of products from this sector. Nonetheless, we should have an open eye for circumstances where return of investments is not always positive for farmers, but may be positive for society as a whole, and discuss possible solutions for such circumstances.

Finally, although we feel that the direction of the research of this White Paper may greatly enhance the quality of animal husbandry and animal welfare, we may expect that farmers and veterinarians who try to do their utmost in establishing an antibiotic-free husbandry will likely face new problems. Their animals may for example have altered nutritional requirements or may suffer from other disorders. Efforts to reduce antibiotic use should not lead to impairment of animal welfare. Also conflicts of interests between different stakeholders may arise. Thus potential bottlenecks and problems associated with antibiotic-free or antibiotic-poor production should be identified and addressed adequately as soon as possible.

Relation with policy and other research initiatives

The principal aim of the policy of the Ministry of Agriculture, Nature and Food Quality in the field of antibiotics is the reduction of antimicrobal resistance. The Ministry therefore pursues the following three objectives:

1. Central registration
2. Gaining an insight into use, trends and developments
3. Benchmarking leading to awareness and responsible antibiotics use on livestock farms and among veterinary surgeons.

For insight into use and effective benchmarking it is necessary to collect data from individual farms at a higher level than the individual farm. This can be realized, for example, within quality systems, at the level of a chain or integration, from a central sector database at the VETbase Foundation or, if necessary, on a sample basis. These objectives have recently been evaluated (Bondt et al 2009). Stake holders appear ready to demonstrate their responsibility for ensuring the responsible use of antibiotics. Private quality systems appear to be a suitable instrument for giving concrete expression to this responsibility. However, the initiatives from the different sectors require further adjustment in order to meet the wishes of the Ministry of Agriculture, Nature and Food Quality in relation to registration. An essential element is a proper monitoring of the use in order to establish whether the implementation of the private initiatives does in fact lead to actual improvements in antibiotics use. To this end, agreement still has to be reached on making the data available to the government in order to give it an insight into the use in the different sectors. It has also been recommended that government makes clear to private parties what data it wants to obtain and what it will do with the data. The initiative of the pig-farming sector, in combination with the initiative of the VETbase Foundation, most completely meets the wishes of the government with respect to central registration and benchmarking. However, It is not clear to what extent the initiative of the VETbase Foundation in itself can count on support among veterinary practices. In the veal calf sector insight into antibiotics use will be obtained through a representative sample in 2010. Within the poultry sector the data of over 50% of the broiler holdings are centrally recorded. The aim is to achieve an increase in the number of participants, including of veterinary surgeons supplying logbook data electronically (Bondt et al 2009).

In addition to policy measures, several research activities have been initiated in the past to address this issue. Naturally this comprehensive White Paper overlaps with some of these activities and will sometimes provide natural follow up. Yet, the focus of this White Paper is at providing an integrated assessment of all aspects of this problem in livestock production therewith avoiding any duplication in research efforts. Relevant initiatives include:

- The ZON-MW program "Priority Medicines Antimicrobial Resistance" (2009-2018), which is primarily focused on public health aspects.
- Activities on antimicrobial drug resistance in the framework of the 7th EU Framework Programme, which includes activities on novel drug targets against Gram negative bacteria, *S. pneumoniae*, biomarkers, nosocomial spread, economic costs, and MRSA.
- Annual monitoring of antibiotic use by the FIDIN.
- Annual monitoring of antimicrobial resistance and antibiotic usage in animals in the Netherlands (MARAN reports) by CVI, LEI, VWA, RIVM, AHS, and FVM.
- Research activities in the framework of the several master plans as described in the "Convenant antibioticaresistentie" (2008), mainly directed at optimizing registration of antibiotic use and measuring the prevalence of resistance (see above).
- Alternatives for Antibiotics (ALTANT), organized by ImmunoValley in commission of the Ministry of Agriculture (2008-2009), mainly directed on alternatives such as bacteriophages, antimicrobial peptides, and phytotherapy.
- The research program ABRES, which is aimed at making inventories of therapeutic use of antibiotics and molecular epidemiology of resistance determinants. Based upon these results further work, as described in this White Paper, should focus on intervention studies directed at specific risk factors.

The next steps: starting and implementing the research

First, government and industry will have to make forward looking choices. Evidently important next steps are to ensure the funding of the research, the formation of a consortium preferably including public-private partnerships that will be responsible for establishing an organization structure and the execution of the actual research, including the drawing up of more detailed plans of work. Obvious partners of the consortium are participants of the Knowledge Chain Animal Infectious Diseases (Kennisketen Infectieziekten Dier, KID) and collaborating private enterprises.

The KID was instituted in 2006 by the Faculty of Veterinary Medicine of Utrecht University, the Animal Sciences Group from Wageningen University and Research Centre - comprising the Central Veterinary Institute, Livestock Research in Lelystad and the Department of Animal Sciences in Wageningen - and the Animal Health Service in Deventer.

The Central Veterinary Institute is the national reference laboratory on the major notifiable diseases and further works together with Livestock Research in Lelystad on strategic aspects of endemic animal diseases. The Faculty of Veterinary Medicine and the Department of Animal Sciences in Wageningen comprise several excellent basic research groups, while the Animal Health Service in Deventer is strong in applied field research and monitoring animal health and diseases based on excellent working relations with veterinarians and farmers in the field. Together they comprise the knowledge infrastructure on animal diseases in the Netherlands.

Major aims of this collaborative network are to join forces, to enhance the scientific basis of animal disease control, to foster knowledge exchange between the partners by linking basic, strategic, and applied research, and to promote scientific innovation as well as the exchange and flow of knowledge from academia to the field, and – equally important- vice versa. In addition, the partners aim at optimizing the use of their complementary expertise and at enhancing the efficiency of their research efforts. Furthermore, by teaming-up the partners aim at increasing their visibility in the field and at optimizing their relationships with government and industry.

While no single institute has the experience to cover all expertise needed, the partners of the KID bring together most of the required knowledge and expertise for this research program. They are in particular suited to cover both the basic and applied parts of the research, have their own niche in the research area and complement each other, and already have an infrastructure that fosters knowledge exchange and translation of results from the laboratory to the field.

For particular purposes, especially with respect to socio-economic and behavioural aspects, and governance issues collaboration with appropriate partners, such as the Social Sciences Group and others, will be established.

The actual daily management of the research should be done under responsibility of a Program Organization, which includes a Steering Committee consisting of Work Package leaders. Progress monitoring and general supervision on behalf of governing bodies and participating partners, including the Ministries of LNV and VWS, stakeholders, and financers should rest with a Board of Supervisors. Where possible the consortium should foster international collaboration and explore the possibilities for establishing intellectual property rights, the valorisation of these, and the further formation of public-private partnerships.

Phasing and prioritizing

Designing a "pathogen-free" animal husbandry or a "disease-free" animal husbandry may have a sustainable impact on the management of pathogens, disease prevention, and hence the reduction in antibiotic use. The research laid out in this White Paper is broad and manifold, and prioritization may therefore be necessary, i.e. a selection of research projects that may offer a large contribution to the solution of the problem. However, this cannot be achieved with great ease, because achieving research outcomes may carry certain risks (genetic selection for resistance, or vaccine development for example), while their potential contribution may yet be very large. Several research activities complement each other, and should be carried out sequentially (for example an inventory of causative factors should precede intervention studies aimed at eliminating such factors). Furthermore, the relative success of certain measures cannot always easy be predicted, take behavioural or management changes for example.

We like to stress that reducing antibiotic use will not be achieved by simple solutions, but require an integrated, multidisciplinary effort of many stakeholders, sometimes necessitating long term and risky research efforts. Nonetheless, we may provide an indication of the time needed in which results may be delivered, i.e. in the short term (1- 3 years), mid term (3 – 5 years), or long term (4 - > 5 years). This has been represented in Table 1.

It is important to stress that both researchers and stakeholders in the field should not wait for each other. Measures to reduce antibiotic use and improve disease control can already be taken immediately without waiting for new alternative drugs, measures to eradicate certain pathogens can already be taken even if the ideal vaccine or diagnostic test is not yet available, and measures to reduce the impact of disease can already be taken even if animals are not yet genetically selected for resistance against a particular disease. It is only to be expected that gradually our instrumentation to support disease prevention will be improved.

Concluding remarks: scientific and societal impact

This White Paper comprises extensive and comprehensive research proposals. However, its scientific and societal impact are likely substantial. Moreover, the urgency to act on reducing and eliminating the problem of antibiotic resistance in animal husbandry and its transfer to the public health sector, while simultaneously improving the quality of animal husbandry and disease management, is immense.

This White Paper therefore in the first place contains research plans aimed at enabling the animal production sector to take up its responsibilities in producing antibiotic-free, hence it is **enabling research**, rather than pure basic or applied research. Scientifically, large progress will be made in the field of endemically circulating diseases of animals, a field that has received somewhat less attention during the last years. Most research efforts described in this White Paper are truly **multidisciplinary**, and indeed scientific breakthroughs are to be expected from cooperation between – for example - nutritionists, gut physiologists, geneticists, and microbiologists when they work together using infection models. In addition we will amply pay attention to dissemination of knowledge aimed at really reducing antibiotic use in the field.

Bacterial and viral vaccine development may benefit greatly from cooperation between microbiologists and X-omics researchers (together comprising the field of vaccinogenomics). Evidently, we may expect to find the research described in this White Paper back in numerous papers, conference proceedings, lectures, courses, education programs, patents, and not in the last place, products, new and optimized animal husbandry systems, quality programs, and changed practices of animal owners, advisors, and veterinarians.

In addition to its scientific impact, the societal impact of this research may be, even should be, very large indeed. Next to its prime aim, reducing antibiotic resistance and antibiotic use in animal husbandry, we consider that the research may allow the practice of animal husbandry, including the field of veterinary medicine, to **"reinvent itself"** by designing the animal production systems of the future. We expect that this research contributes to an animal production sector that is of higher **quality**, without preventive use of antimicrobials, and is therewith more societal acceptable. Hence, is it will reconfirm its **Licence to produce**.

Furthermore, animal health care will become of a higher quality, more challenging, and therefore more interesting to work in. We consider that much of the research efforts will contribute to more **sustainable preventive solutions**, i.e. without shifting negative consequences to other segments of society or future generations.

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Table 1. Phasing of research results

Research aim	Results deliverable in short term (1- 3 years)	mid term (3 – 5 years)	long term (4 - ≥ 5 years)
Reasons, causes, and factors • Establishing and verifying which diseases and pathogens are responsible for most antibiotic use. • Monitoring temporal changes in the occurrence and characteristics of causative pathogens, in particular their potential changes of virulence markers and antigenic composition that may hamper vaccine effectiveness. • Defining the epidemiological, evolutionary, and pathophysiological mechanisms that determine the occurrence and severity of endemic infections, including those of resistant bacteria. • Identifying which factors (including aspects of antibiotic use) predominantly lead to emergence, spread and possible maintenance of resistant bacteria and resistance markers. • Establishing the role of animal movements in the production chains (i.e. the pyramid structure) and management factors that affect infectious pressure and spread of resistant bacteria and resistance markers	+++	++	+
• Characterize ill-defined disorders with respect to aetiology, pathophysiological mechanisms, and causative (co-)factors. Define them precisely. • Such research should especially be directed on finding clues for preventive measures. Measures have to be evaluated on effectiveness, cost benefit ratio, ease to implement, and reliability.		+	+
• Establish the reasons (diagnoses, arguments, incentives, management disorders) for antibacterial use. Therewith define the major reasons for farmers and veterinarians to decide to treat animals with antibiotics. • Examine differences between farmers in antibacterial use. Therewith take socio-economic, behavioural, and attitudinal aspects that may determine antibiotic use into account. • Relate management factors (e.g. housing, group size, climate control, import of animals, all-in all-out systems, age and method of weaning, nutrition, behavioural factors etc) with the quantity of antibiotic drug use on farms, explain differences between farms, and define “best practices” for a sparse use of antibiotics.	+++	++	
Pathogen-free design: eradicology			

Research aim	Results deliverable in short term (1- 3 years)	mid term (3 – 5 years)	long term (4 - ≥ 5 years)
• Develop a theoretical framework employing a multidisciplinary systems design approach that may underlie a pathogen-free animal husbandry. • Provide a comprehensive overview, employing risk analysis, knowledge of population dynamics of endemic pathogens, and cost-effectiveness calculations, of the pathogens for which eradication at herd or regional level may be feasible at a wide scale. Examples may include PRRSV, Actinobacillus pleuropneumoniae, Haemophilus parasuis, Mycoplasma spp., Pasteurella multocida, Bordetella bronchiseptica, Brachyspira hyodystenteriae, Lawsonia intracellularis (pigs), Salmonella gallinarum, Mycoplasma gallisepticum, Ornithobacterium rhinotracheale, and Brachyspira spp. (chickens). • Formulate preconditions for eradication of selected pathogens at herd level. • Formulate how the structure of the animal production sector and internal management systems at farms should be employed or optimized to support and maintain pathogen eradication. • Optimize methods of eradication and provide evidence of their effectiveness. • Perform feasibility studies to eradicate selected pathogens on a wide scale at herd level. Consider the possible need to eradicate resistant bacteria. • Formulate requirements for supportive diagnostics and vaccines.	+++	++	++
Disease-free design: lowering the incidence and impact of infections • Understand and optimize management factors that may lower the infectious pressure on farms, or that may optimize the animal's resistance and resilience to infection, in particular during stressful events. • Identify and study immune modulatory effects that are affected by management measures and that influence infectious disease resistance. • Define stressful transition periods during the production cycle during which the animals are most susceptible for disease development (e.g. weaning, transport), identify the causative pathophysiologic mechanisms, and develop methods to prevent disturbances of homeostasis, and/or to support and improve the relevant biological processes (e.g. the application of specific immune modulators (pre-/probiotics) during weaning). • Explore the ability of young animals (protected by maternal antibodies) to develop long lasting protection after exposure to low numbers of pathogens. • Provide evidence of the effectiveness of management interventions and new system designs.	+	++	++

Research aim	Results deliverable in		
	short term (1- 3 years)	mid term (3 – 5 years)	long term (4 - ≥ 5 years)
• To provide further proof of principle of genetic approaches to enhance disease resistance. • To integrate genetic and pathophysiological data on particular infectious diseases to identify relevant genes, gene variants and molecular pathways that can be used as a target to improve animal health by genetic as well as non-genetic approaches. • To establish host gene-pathogen gene-environment interactions. • To increase our understanding of the various molecular genetic pathways that animals use to resist establishment of infection or to avoid developing clinical symptoms by comparing high and low susceptible selection lines by functional genomic approaches. • To elaborate on the new tools of genomic selection (including 60.000 SNP chips) to develop practical ways to select against disease susceptibility and the development of clinical symptoms upon infection. • To improve the accuracy of genomic breeding value predictions with respect to phenotypes related to infectious disease and the use of antibiotics. • To develop an animal trait ontology in the field of livestock infectious diseases.		++	+++
Health-promoting nutrition • Development and application of experimental infection models, either using mono- or multi-infections, in which the influence of nutritional factors on clinical and microbiological outcome parameters of infection, and their modes of action, can be examined. • Understanding of the mechanisms by which feed constituents affect infectious diseases. • Development of measurable parameters of gut function and microbial homeostasis. • Development and application of field study protocols, for example guided by principles as used in randomized, double-blind, placebo-controlled and cross-over studies, to unequivocally allow demonstrating the effect of nutritional factors on clinical, microbiological, and economic parameters under field circumstances.	+++		
• Providing a sound understanding of gut function and its interaction with bacterial colonisation by normal and pathogenic microbial flora, particularly in young animals and especially around transition periods (e.g. the time of weaning). • Identifying improved feeding strategies and bioactives, and their modes of action, as alternatives to the use of antibacterial medicines as prophylactic treatments and growth promoters.		++	++

Research aim	Results deliverable in short term (1- 3 years)	mid term (3 – 5 years)	long term (4 - ≥ 5 years)
• Develop test systems to select promising strategies that positively influence the gut's resistance to infection. • Determine intestinal effects of various probiotics and prebiotics. • In particular, examine and optimize the use of microbial populations (starter cultures, probiotics) as means to establish a healthy and stable microbial population in the gut.		++	
• Develop strategies at the end of the lactation period to reduce intramammary infections and the need to use antibiotics. Provide evidence of their effectiveness. • Understand the mechanisms by which nutrition and feed ingredients affects the udder's resistance to infection.		++	
Better diagnostics • To develop and evaluate rapid, robust, and cheap diagnostic (multiplex) tests that are able to guide therapy and prevention under field conditions, thus refining, reducing, or replacing the use of antibiotics. • To include rapid tests for virulence and antigenic (serotype) markers that may be relevant for installation of therapy, prevention, or surveillance purposes.		++	
• Developing rapid tests for antimicrobial resistance markers that are useful for therapeutic and surveillance purposes.	+	++	
• Development and validation of diagnostic tests of host parameters that may be useful in the early detection of health disorders. • Definition of their added value in disease prevention and control.		++	+++
Better vaccines and vaccinations • Design optimal preventive vaccination programs for swine, poultry, and calves under field conditions. Therefore develop tools that allow farmers and veterinarians to design and optimize strategic vaccination plans that are pro-active, (cost-)effective, based on a sound risk analysis, and aimed at minimizing disease and antibiotic use. • Develop tools to optimally evaluate (cost-)effectiveness of vaccination strategies under field circumstances. • What are limitations in combining vaccine antigens and how can these be circumvented? • Identify impediments, whether technical or socio-economic, to implement optimal vaccination strategies.	++	++	++

Research aim	Results deliverable in short term (1- 3 years)	mid term (3 – 5 years)	long term (4 - ≥ 5 years)
• How can we rapidly identify immune mechanisms responsible for protective immunity? • How can induction of protective immunity be optimized, especially at mucosal sites and directed at inactivated vaccine antigens? In particular, how can pathways of antigen recognition and presentation be optimally activated by new types of adjuvants or immune potentiators? How can we selectively stimulate essential immune pathways with a minimum of adverse reactions? • How can immune suppressive and immune escape mechanisms (including low immunogenicity) of pathogens be circumvented? • How can we circumvent serotype-specific immunity? How can we prevent adaptation or escape of bacteria from vaccine-induced immunity? How can we optimally adapt vaccines to changing pathogens? What should we know about pathogen evolution in vaccine development? • How can we optimize and use genomic tools to rapidly identify and select candidate vaccine antigens?		+	+++
• How large is the influence of genetic variation on the magnitude and efficacy of vaccine-induced immune responses? • Which polymorphic host genes and genetic pathways determine the effectiveness of vaccine-induced immune responses? • How can vaccination programs be optimized to specific breeds or genetic backgrounds? What are optimal gene-vaccine combinations?		+	+++
Develop or improve vaccines against: • PRRSV. Although a viral disease, PRRSV causes reproductive disorders in sows and enhances sensitivity of fattening pigs to several respiratory bacterial pathogens. It is therefore responsible for a large antibiotic use. • *S. suis* in pigs. • Porcine post weaning diarrhoea/oedema disease. Although optimization of weaning may reduce the incidence of this disease complex, a vaccine may further substantially reduce antibiotic use of pigs during this period. • Swine dysentery, caused by *Brachyspira hyodysenteriae*. • Necrotic enteritis in poultry, caused by *Clostridium perfringens*. • *Ornithobacterium rhinotracheale* in poultry. • *Avibacterium paragallinarum* in poultry. • Colibacillosis in poultry. • Respiratory disease complex in calves, and respiratory disease complex in pigs, both caused by a multitude of pathogens. Combination vaccines optimized to local and current circumstances may be of great benefit. • MRSA.		++	+++

Research aim	Results deliverable in short term (1- 3 years)	 mid term (3 – 5 years)	 long term (4 - ≥ 5 years)
Alternatives • To identify novel molecular pathways as new targets for intervention in bacterial infections. • To develop new (high throughput) selection systems (in vivo, in vitro) for selecting new drugs without the "current" disadvantages. • To formulate requirements for alternative drugs based on a risk assessment approach. • To develop test models in which the effects of novel, alternative treatments on microbiological, clinical and economic outcome parameters following infectious challenge can be established and validated.		+	++
• Explore the possibilities of phage therapy for use in animals. In particular select a few infectious diseases (for example swine dysentery, weaning diarrhoea, S. suis) that may benefit from phage therapy. Take consequences for public health into account. • Develop application methods (mixes, sequential application of phages, etc) that circumvent phage resistance or minimize its consequences. • Explore the possibilities of optimizing phage therapy, for example by genetic improvement of phages.	+	++	
Implementing changes • Define which conditions have to be fulfilled to stimulate reduction in the use of antibiotics. • Get insight into the timing and order of the implementation of the different measures. • Make an inventory of impediments that should be overcome, for example lack of knowledge among farmers on correct use and long-term consequences of antibiotic use, or economic constraints. • Examine which positive and negative incentives may be supportive in establishing a cultural change, i.e. in changing attitude and behaviour towards the use of antibiotics. Examples may include benchmarking, higher prices for farmers that produce without antibiotics, taxes on antibiotics, and division of responsibilities regarding diagnosis of disease, prescription of antibiotics, and sale of antibiotics. • Get insight whether differences between farmers needs different approaches. • Examine governance and management aspects aimed at reducing antibiotic use both at national and international levels. Take experiences in other countries into account. Take the potential contributions of national and international governing bodies, and professional, retail and consumer organizations into account. • Therewith define the best governance practices to reduce antibiotic use in animal husbandry. • Examine how other stakeholders, such as the meat and milk industries, retail organizations, and consumer organizations, may be involved by demanding or promoting a certified and guaranteed antibiotic-free or antibiotic-poor way of producing.	++	++	

Research aim	Results deliverable in short term (1- 3 years)	mid term (3 – 5 years)	long term (4 - ≥ 5 years)
• Optimize knowledge transfer from the scientific domain to the field. • Explore the possibilities of farmers' innovation networks to act as forerunners and examples, and examine how such networks should be supported so that their results can be optimal and that their experiences can be disseminated quickly to the broader agricultural community. • Provide evidence of the effectiveness of suggested measures aimed at establishing shifts in attitude and actual behaviour towards the use of antibiotics.			
Registration and monitoring of antibiotic usage and resistance • Developing, optimizing, and harmonizing methods for quantification of resistance in populations (e.g. farm level, national level). • Optimizing methods for quantification of antibiotic usage in populations. • Identification of risk factors for antibiotic usage for respectively high and low levels of resistance. • Relating trends in antibiotic use with trends in antibiotic resistance, including the potential transfer of resistance from animals to humans. Preferably establish causative relationships. • Optimizing methods of providing animal owners, advisors, veterinarians, and policy makers with feed-back data. • Developing tools for metagenomic analysis of resistance genes in different matrices enabling a study of transmission routes of resistance in the environment, surface water, food chain, etc.	++	++	++
Communication and scientific exchange • Organize and foster multidisciplinary working groups of researchers. • Enable scientific communication through joint meetings. • Ensure that results are communicated to the scientific community. • Promote collaboration and information exchange across Europe. • Speed up the process of knowledge exchange from basic research to applied research to policy makers and field workers, and vice versa.	++	++	++

+, ++, +++ Relative measure for research output.