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## The Applicability of Animal Health Surveillance Systems for Post-Market Monitoring of Potential Adverse Effects of Genetically Modified (GM) Feed.

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### ABSTRACT

A facultative post market monitoring of potential health impacts of genetically modified (GM) feedstuffs on livestock consuming these feeds after pre-market risk assessment is under ongoing consideration. Within the IPAFEED database, scientific studies on health effects beyond performance in livestock and the results of a systematic search for evidence of outcome effects due to GM feed are consolidated. These outcomes were reviewed and checked for consistency in order to identify plausible syndromes suitable for conducting surveillance. The 24 selected studies showed no consistent changes in any health parameter. There were no repeated studies in any species by GM crop type and animal species. As such, there is insufficient evidence to inform the design of surveillance systems for detecting known adverse effects. Animal health surveillance systems have been proposed for the post market monitoring of potential adverse effects in animals. Such systems were evaluated for their applicability to the detection of hypothetical adverse effects and their strengths and weaknesses to detect syndromes of concern are presented. For known adverse effects, applied controlled post-market studies may yield conclusive and high-quality evidence. For detecting unknown adverse effects, the use of existing surveillance systems may still be of interest. A

simulation tool developed within the project can be adapted and applied to existing surveillance systems to explore their applicability to the detection of potential adverse effects of GM feed.

KEYWORDS:

Genetically modified crops, Animal feed, Animal Health, Surveillance, Post Market Monitoring (PMM).

HIGHLIGHTS:

- No evidence for health impacts of GM feeds on livestock exists to allow the development of a surveillance system targeted specifically at such known adverse effects.
- For detected previously unknown adverse effects tests to determine any potential association with GM feed are limited, as such, the use of animal health surveillance for detection of adverse effects has significant weaknesses.
- Type I and type II errors, *i.e.* the possibility of failing to detect an adverse effect which was present and the possibility to detect adverse effects which are not truly present, would compromise the utility of any post-market monitoring system for GM feed.
- The current terminology used in GM feed post market monitoring is not consistent with terminology generally used in animal health surveillance.
- The cost-effectiveness of any surveillance approach for GM feed adverse effect is likely to be poor, due to a high cost per animal detected and there is therefore further work in this area required.

ABBREVIATIONS:

BIP, border inspection post; EU, European Union; GM, genetically modified; GMO, genetically modified organism; OIE, World Organisation for Animal Health; TRACES, Trade Control and Expert System

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## 1. Introduction

This paper summarizes the context in which the MARLON project consortium has developed an approach to inform monitoring livestock for potential adverse health impacts resulting from the consumption of animal feeds derived from genetically modified (GM) crops. The acronym MARLON stands for “Monitoring of Animals for Feed-related Risks in the Long Term”. The three-year MARLON project was funded by the European Commission under its Seventh Framework Program for research and technology development.

One of the main goals of the project was to provide data and approaches that could assist with the design of a post-market monitoring program for detection of possible adverse health effects in livestock of specific GM crops being sold within the European Union (EU), in case there was a requirement to do so. At the time of project initiation (2012), such a requirement did not yet exist for any of the GM animal feeds that had already gone through the mandatory regulatory approval procedure. This procedure is mandatory for all GM foods and feeds before permission can be granted for their market entry within the EU. Part of the procedure is a pre-market safety assessment, which may identify also outstanding issues that warrant post-market monitoring. This could then become a requirement as part of the EU authorities’ decision to allow the GM crop and its derived animal feed onto the market. The development of these tools was therefore of a prospective nature.

Towards this end, MARLON brought together the different disciplines of regulatory safety assessment of genetically modified organisms (GMOs) and veterinary epidemiology. Whilst the first was borne out of a precautionary approach as there were no hazards known to be

inherently linked to genetic modification, the latter usually focuses on known hazards such as microbial pathogens or on production outcomes. Moreover, the safety assessment of GMOs usually entails experimental studies performed under controlled conditions, whilst the epidemiological approaches to post-market monitoring would utilise data generated by animal health surveillance systems. In order to explore what options are feasible, the project considered the background knowledge on potential health impacts of GM-crop-derived feeds, the possibility to measure exposure of livestock to such feeds, the structure of the various feed and livestock production chains and the existing health surveillance schemes.

Here, after a summary of the recent history of the introduction of GM crops into the EU market, an overview of the various kinds of animal health surveillance systems is being provided. It is considered how the latter can be designed and adapted for the purpose of monitoring GM feeds for their potential adverse health impacts. Moreover, we explored the published literature and data from published livestock feeding studies in the IPAFEED database established within MARLON. The purpose was to verify if there are any animal health and production parameters that could be used in surveillance for potential adverse health effects specifically linked to GM feed intake. While the project specifically focused on GM feeds and production chains and regulatory context within the EU, the applicability of the project outcomes can easily be extended to other feeds and beyond the EU.

## 2. Background

### *2.1. GM-crop-derived feeds*

Over the past two decades, following the commencement of the large-scale cultivation of genetically modified (GM) crops obtained through modern biotechnology techniques in the mid 1990s, the adoption of these crops has expanded continuously, reaching a global acreage of 181.5 million hectares worldwide growing these crops in 2016. Most of these are commodity crops such as soybean, maize, cotton, oilseed rape, and rice, whilst the most popular traits introduced through genetic modification include herbicide resistance and pest insect resistance. While there are 18 countries growing more than 50,000 hectares of such crops (1), it is conceivable that, through international trade in raw agricultural commodities or derived products, other countries not growing GM crops or only to limited extent may still import sizable amounts of these commodities. These could be used, for example, as ingredients for the production of animal feeds, such as from maize gluten or oilseed (soybean, canola, cottonseed) meals.

### *2.2. Regulatory pre-market safety assessment & post-market monitoring of animal feeds from GM crops*

Before GM crops and food and animal feed products derived from these crops can be sold, many countries require that they undergo a regulatory procedure which also entails a pre-market safety assessment. For such assessments, the internationally harmonized approach of comparative assessment is applied, which has been enshrined in guidelines for such safety assessments published by the authoritative Codex Alimentarius, of which most



nations of the world are members (2), and further elaborated by others, such as the EFSA GMO Panel. According to this approach, an extensive comparison of a GM crop with a genetically close non-GM comparator with a history of safe use will help identify intended and unintended changes brought about by the genetic modification. As such, an extensive analysis of molecular, compositional and phenotypic/agronomic characteristics is commonly undertaken, after which any differences found are evaluated for their relevance and it is subsequently decided whether the information base including any parameter changes are sufficient to conclude that the risk of potential safety implications requires additional information in the form of monitoring or additional tests (for example, animal toxicity tests).

Whilst in many cases pre-market assessments are sufficiently conclusive, it can be envisaged that in some specific cases, it may be desirable to collect further data in the post-market stage. This could serve the purpose of verifying whether any assumptions made during the pre-market assessment, such as for consumption of the product, need to be adjusted or checked for hazards identified during the pre-market assessment. Under EU legislation(3) for example, decisions for the regulatory approval of a specific GM product may include a clause requiring such case-specific post-market monitoring for a particular feature, such as consumption estimates or a hypothetical adverse effect. So far, such requirements have particularly been imposed for GM oilseed crops with modified fatty acid composition, for which applicants seeking to market them were obliged by the decisions approving their commercialization to verify the consumption in subpopulations given the possible impact on human nutrition. Such requirements have not been imposed yet by the EU for animal feed specifically. Besides the optional case-specific monitoring for possible impacts of specific GM crops, applicants in the EU have to perform general surveillance for possible unforeseen

health and environmental effects of such crops and their derived products for all GM crops. The European biotechnology industry association EuropaBio, for example, has elaborated a procedure towards this end. It involves the associations of operators dealing with imported viable commodities, which are to be informed of approvals of GM crops that are subject to the requirement of surveillance, and reminded to report any unexpected adverse effects resulting from the consumption of these ingredients (4).

Also outside the EU, regulatory authorities in charge of safety assessment of GM foods and feeds consider the possible need for post-market monitoring under specific circumstances. Food Standards Australia New Zealand, for example, notes that the pre-market safety assessment already provides assurance and considers general passive surveillance impractical, particularly in the absence of a specific hypothesis. It also refers to specific scenarios under which case-specific monitoring could be helpful, such as for nutritionally altered GM crops, to verify assumptions made during the risk assessment regarding nutritional and health impacts of their consumption on the population (5). In the United States, the Centre for Food Safety and Applied Nutrition's Adverse Event Reporting System coordinates their passive surveillance system. This system allows consumers to report suspected adverse reactions; almost 100,000 self-reported records of events related to food consumption were reported within the period January 2004 – June 2017. Few of these reports refer to GM foods. Although it is not clear from these records if and what follow-up was conducted in response to these reports, it shows that GM foods and feeds are also considered to fall within the scope of these reporting systems (6).

### *2.3. The MARLON project*

For the purpose of developing a harmonized approach which applicants could employ to establish case-specific monitoring initiatives, if required by EU decision makers, to check for specific health impacts of a particular GM feeds, the EU-funded MARLON research project was initiated in 2012 (7). Within this three-year project, various factors that could enable the establishment of a post-market monitoring initiative were explored, such as background knowledge on possible health impacts of GM feeds in livestock, insight into the organization and information flow within animal production chains and the possibility to measure exposure of livestock to GM feed ingredients as well as indicators for four pre-identified health impacts. Importantly, the project also sought to develop a simulation tool that could assist applicants in assessing whether a certain hypothetical adverse effect on animal health could trigger a trend deviating from the background pattern of 'normal' health and production parameters taking into account a range of factors and actors, such as the responsiveness and actions of farmers, veterinarians, reporting systems, diagnostic laboratories, as well as the specifics of the organization of the livestock production and health monitoring systems. Such a probabilistic tool would help to assess the ability of a system based on existing animal health and production data to detect a hypothetical effect in the livestock population of interest.

### 3. Animal health surveillance and its applicability to GM feed post-market monitoring.

The approach to be developed within MARLON had to focus on assessing the usefulness of existing surveillance systems for animal health. Such systems, in a more general sense, are an ongoing requirement for animal trade and production management (8), whilst none has been specifically developed to assess potential adverse health impacts of GM feeds. The data generated through national surveillance systems are able to support policy decision development regarding animal health management. National systems also provide evidence of disease freedom and early detection of outbreaks for national and international organizations (9). The process of surveillance consists of data collection, analysis and interpretation; and the outputs are used to inform decisions on animal and public health prevention interventions (*e.g.*, (10, 11)). The potential use of existing animal health surveillance systems for monitoring of GM feed adverse effects has been discussed (12, 13). These authors recommend involving farmer and producer organizations into sentinel surveillance networks and have also developed sample questionnaires for reporting of a broad range of generic health conditions. They also acknowledge that monitoring indicators will depend on the pre-market risk assessment pathways and knowledge gaps (12). We have reviewed current practices of post-market monitoring of biotechnologically enhanced feed stuffs, attempted to find any evidence for indicators which may have higher risk. With a view to adapting existing animals health surveillance systems to include GM feed adverse effect detection.

#### *3.1. Overview of types of surveillance used in animal health*

Many animal health surveillance systems are in place, the functions of these include, providing evidence of disease freedom, early detection of disease outbreaks, and estimation of the prevalence/incidence of endemic diseases. Broadly, surveillance can be categorised based on the

objective of the system. The accuracy of the surveillance system will vary depending on the number of animals (or farms) observed, the diagnostic test used and the statistical confidence level required. For example, an animal health system which aims purely to determine the prevalence of an endemic disease will be set up entirely differently from a surveillance system aimed at establishing freedom from disease or early warning of an emerging concern (10). Below, we review the different types of surveillance systems that might be of relevance for the purpose of providing input to post-market livestock health monitoring initiatives.

### **3.1.1. Syndromic systems**

A syndrome is a group of symptoms and signs that collectively characterize a particular disease. Syndromes are therefore usually non-specific, in contrast to a laboratory confirmed diagnosis. Syndromic surveillance can have a variety of purposes, such as detection of emerging, possibly unknown threats to animal health, early warning at the start of an unexpected event, situational awareness of threats on a day-to-day basis, and reassurance of a lack of impact of known health threats (14).

Syndromic surveillance systems are based on regular timely reporting of symptoms associated with a specific syndrome. These reporting activities can be either by lay reporting or veterinary reporting and are usually passive in nature (15). Syndromic surveillance relies on detailed historical data analysed to ascertain the expected level of occurrence. An alarm is triggered when reporting exceeds expected levels, this in turn allows for animal health professionals to investigate the cause of this alarm (15).

A recent review by Rodriguez-Prieto and co-workers systematically investigated the scientific literature on animal health syndromic surveillance systems (16). This followed a previous review in

2011, which had concluded that, at that point in time, the field of syndromic surveillance was starting to grow fast. In particular, the review considered the different data sources used as inputs, animal populations being surveyed, and the particular system's stage of development. It also considered published studies on statistical methods and detection of disease outbreaks that can be applied for this purpose. The various data sources identified were categorized as follows:

- Production data: For example, milk production and reproduction in cattle;
- Clinical data (primary data): For example, syndromes reported by veterinary practitioners;
- Clinical data (secondary data): For example, carcass necropsy findings;
- Laboratory data: For example, laboratory analyses of pathogens in samples collected;
- Mortality data: For example, weekly reports from national animal registries and renderers;
- Abattoir data: For example, periodic reports on carcass condemnations and types;
- Media sources: For example, media reports on livestock disease outbreaks;

Interestingly, the EU-funded Triple-S project published its guidelines for the design and execution of syndromic health surveillance plans in 2013, covering both human and animal health (14). It considers the following stages in the development of a syndromic surveillance system:

- Setting up a system, based on a needs assessment (*e.g.* data gaps, availability of syndrome data from existing data sources), definition of purpose and possible synergies with existing systems, assignment of roles and tasks to staff and contacts, operational and legal/governance provisions, and resource planning.
- Collecting data, and preparing them for analysis, based on identification of data sources (*e.g.* veterinary clinics, slaughterhouses, laboratories, professional networks, pharmaceutical sales, tele-health reporting, passive reporting, renderers, national livestock registries), selection of variables to be collected (with a distinction between denominator and numerator, such as population size and number of symptom reports within a given time interval; including, for example, animal species, demographic, geographical and temporal

data preferably also with indications of severity), data coding, data recording, transmission and centralization, and data aggregation. Also important are ensuring continuity of data access, and safeguarding confidentiality, security, and quality of the data.

- Analysis of data, distinguishing between prospective analysis, *i.e.* early event detection and reassurance of no health impact, and retrospective analysis, *i.e.* *post-hoc* quantification of health impacts allowing for the use of more complex calculation methods, also taking into account various internal and external factors. The following key steps can be discerned: 1) preparation of aggregated data as indicators (absolute or relative) for statistical, epidemiological analysis; 2) choice and application of appropriate statistical methods taking into account purpose, data characteristics and accuracy; 3) checking of data for epidemiological relevance (*e.g.* contact data source, compare with data from other systems, verify if other statistical methods would have yielded different outcomes); 4) risk assessment, including a characterization of the threat and public health impacts; 5) translation into public health measures.
- Communication of the outcomes to different groups of stakeholders, such as decision makers, surveillance system participants, actors taking part in other surveillance systems, and the general public (15).

From these considerations, it becomes evident that the establishment of syndromic surveillance systems requires organizational measures beyond the actual collection, processing, and interpretation of data. This also by and large holds true, in more general terms, for the other types of surveillance discussed in the subsections below.

### 3.1.2. Risk-based surveillance

With this approach, the data collection will be stratified, taking into account existing knowledge about variation of disease risk within the population. This will guide the planning, design, and

implementation of surveillance plans. The knowledge on risk variation can be based, for example, on historic data, risk profiles, or outcomes of modelling exercises. The identification of factors that impact risk can be supported by different types of evidence, including epidemiological studies, opinions elicited from experts, and risk assessments (17). Factors that may indicate increased or decreased risk include, for example, veterinary health checks, trade and long-distance movement of livestock animals, farming intensity and other husbandry practices and farm characteristics, geographical location, and animal age (infectivity window). An appropriately designed risk-based surveillance should achieve at least the same likelihood of early detection of disease while improving the cost-effectiveness of the surveillance activities.

Risk-based surveillance may utilise active and/or passive surveillance approaches. A study commissioned by the European Food Safety Authority's (EFSA) GMO Panel has worked on describing the probability of detecting a GM crop-related adverse effect on plant and animal health through active reporting systems involving networks of farmers and producers throughout the production chains. The authors concluded that adequate sensitivity would be difficult to achieve for rare adverse events even if there were great awareness, representativeness and coverage of the samples taken (12). The benefits of a passive system are that it is cheap and constantly ongoing in the background. The pitfalls are that use of the system is non-specific, sampling is delayed until after a concern has been raised, and under reporting is common (18). Another potential limitation of using a passive surveillance system is that data are lacking to support the relationship between the health outcome and a specific GM product. (19, 20).

Interestingly, the EU-funded RiskSur project developed a guidance for best practice towards risk-based and cost-effective animal health surveillance systems. This was done in preparation of the implementation of the new EU Animal Health Law (21), which attaches great value to information gathering such as surveillance to inform eradication, control, contingency planning, and awareness



of diseases. The EU Animal Health Law also created more flexibility for EU member states with regard to risk management measures, of which surveillance was supposed to constitute an important part, particularly in order to allow for risk-based and cost-effective monitoring (17). It can be envisaged that risk-based approaches also have value for other global regions outside the EU.

The RiskSur best practice guidance acknowledges that prioritization is a key initial step in the process of establishing risk-based surveillance (17). This will consider the wide-ranging impacts of animal disease on, for example, animal health, animal welfare, public health, and societal, economic, and environmental aspects. Besides the data and experts' opinions on these impacts, it also needs to consider decision makers' and other stakeholders' preferences. Planning of surveillance to identify prioritized hazards should include evaluation of the applicability of the existing surveillance systems to provide such data.. Once it has been decided to establish a surveillance scheme, its design needs to comprise one or more surveillance components, each of which focuses on the occurrence of clearly defined cases (17).

The design should consider the hazard, population at risk, spatial and temporal context. This design may be different depending on the specific purpose of the system. Data collection may be performed in a risk-guided manner through weighting the sample size calculation by the risk to reduce overall sample size, and by selectively sampling a given proportion of the total quantity through selectively targeting higher-risk strata. If risk-ratios are known, they can be used to infer the overall disease prevalence from the risk stratum specific observations (22, 23).

Implementation requires clear assignment of roles and responsibilities, and awareness raising amongst stakeholders who can provide inputs to the system. Data management measures and information needs to be in place to collect and process data, while reporting practices need to be established both internally and externally with, for example, policy makers and the general public.

Finally, the surveillance activities should be subject to evaluation, which in turn has to be performed in a cost-effective and efficient way. To this end, RiskSur had developed a tool (EVA-TOOL) that guides the formulation of questions. Evaluation can take place at different stages of the surveillance undertaking and can be triggered by changes in the context, such as local disease outbreaks, changed control options, and public health issues. The final surveillance plan should document all features with sufficient level of detail as recommended by the World Organisation for Animal Health (OIE), for example purpose, populations, resources, activities, roles and responsibilities, end-products, the supporting information system, dissemination & reporting, and evaluation of the surveillance system (17).

### **3.1.3. Surveillance based on data collection point**

Examples of collection points for this type of surveillance include abattoir surveillance, market surveillance, and dip tank surveillance. These modalities are commonly used for infectious diseases and public health concerns to reduce the spread of zoonotic disease and early detection of emerging conditions. These collection points fit into both active and passive surveillance schemes. Abattoir inspections, for example, have the advantage that they are relatively affordable, commonly carried out in all except for the smallest abattoirs, and performed on large numbers of animals, whilst these inspections are done by skilled staff. Drawbacks are that the data may be biased by, for example, samples that are non-representative for the whole population and selection of certain carcass parts for inspection. Moreover, certain lesions and observations may be caused by multiple conditions. Abattoir data could therefore well fit into syndromic surveillance schemes described above (19).

### **3.1.4. Checkpoint / quarantine / export station surveillance**

The purpose of this type of surveillance is to ensure detection of notifiable diseases prior to export or import. The Terrestrial Animal Health Code and Aquatic Animal Health Code of the OIE stipulate

that member states should ensure that border posts and quarantine stations (frontier posts for aquatic animals) are organized and equipped with a veterinary service. This should endow the posts with the ability to inspect incoming live animals and carcasses for diseases, detect and isolate diseased animals, and carry out disinfection and disinfestation (24, 25).

The EU, for example, has to approve “veterinary border inspection posts” (BIPs) within the EU and associated countries (Iceland, Norway, Switzerland). BIPs are located at points of entry into the EU at airports, ports (canal, river, and sea), roads, railways, and inside the country. For the import of live animals from outside these European countries, a health certificate, *i.e.* the Community Veterinary Entry Document, has to be issued by the centralized TRACES database (Trade Control and Expert System). The documents and the animals themselves will be checked at the BIP by an official veterinarian, while additional checks may also be performed by a veterinarian at the final destination. Besides live animals (livestock, equids, companion, laboratory animals, etc.) and carcasses, border inspections may also cover imported foods and feeds of animal origin, animal-material-containing waste from international means of transport, and animal feeds. Issues considered include animal health, animal welfare, and veterinary public health (26). Results of veterinary checks at BIPs will be reported through TRACES, which allows, for example, for monitoring of health and quality of consignments under scrutiny (*e.g.* meat from countries for which mandatory checks have been imposed due to health or safety concerns. It can be envisaged that such data on checks, including the reasons for rejection or detention, would be amenable to inclusion in surveillance schemes, such as for syndromic surveillance discussed above.

A parallel investigation within the MARLON project reviewed the organization of various animal feed and livestock production chains in Europe, including also the various types of inspections and controls, such as BIPs. The purpose was to explore whether data on the presence of specific GM feed ingredients can be traced throughout the production chain and which animal health checks and

controls would lend them for use during surveillance. It was concluded, among others, that within the EU, legislation has enabled the tracing of livestock for the purpose of animal identification and monitoring for infectious diseases (27).

### 3.1.5. Sentinel herd / flock - sentinel surveillance

This type of surveillance relies on identifying specific herds or flocks which may act as pre-warning of the disease. For sentinels used for surveillance of infectious animal diseases, important in the choice and use of a sentinel is its response to the hazard (pathogen in case of infectious disease): The sentinel should be susceptible to the same disease as the total population of interest. Sentinel populations are often, although not exclusively, populations for which the probability of detecting the outcome of interest is increased. This may be due to a higher exposure to the hazard or pathogen, or more rigorous monitoring (28).

Translated to the hypothetical scenario of surveillance of livestock for health impacts of GM feeds, sentinels could be animals which may be more susceptible or which consume consistently more GM feed.

### 3.1.6. Representative survey

A representative survey is a probabilistic survey which is often used to establish disease prevalence performed as an active surveillance activity. Achieving the desired level of certainty requires a sufficiently large sample size, which can be impractically large for low prevalence conditions if a small relative error is required. The bias introduced by imperfect tests or means of ascertaining disease status should be considered and the apparent prevalence adjusted on the basis of the sensitivity and specificity values to obtain the likely true prevalence.

### *3.2. Evaluation of surveillance systems*

The evaluation of surveillance systems helps to establish the level of certainty with which either a region can be defined as disease-free or the bounds (range) of prevalence can be estimated. The strength of the confidence in this range can also be calculated and expressed based on the size of the sample and assuming it is representative of the reference population. For example, an outcome could be “95% confidence that the prevalence of the disease syndrome in the target population is 5% with a precision of +/- 1%.” Furthermore, with a pre-defined level of precision and confidence required, it is possible to calculate the number of farms and animals which would need to be monitored while considering variables such as the risk of a disease occurring.

#### **3.2.1. Sensitivity, specificity and misclassification**

The sensitivity of a surveillance system is the ability of that system to correctly detect animals as positive given that they have a syndrome. Similarly the specificity of the surveillance system can be defined as the ability of the system to correctly detect animals as not having the disease given that they are healthy. In the context of post-market monitoring of potential adverse effects of GM feed, the sensitivity can be defined as the probability of this system to detect an abnormal health effect given that a true abnormal health effect has occurred. Whilst, the specificity of the system can be considered as the proportion of instances where the system will detect only truly abnormal health effects, and not cases which may be attributable to other conditions. Misclassification can occur where a system fails to identify an abnormality as abnormal, or where a normal parameter is wrongly classified as abnormal. This can be due to bias in sampling, measurement error, test interpretation etc. This misclassification will impact the system sensitivity and specificity for detecting abnormalities.

### 3.2.2. Errors to consider in animal health surveillance

The risk of type I error is the probability that the surveillance system produces information that leads to the making of a decision when the real state of the population would not require it. That is, Type I errors imply that decisions are unnecessarily made leading to unnecessary expenditures. For example, in the context of monitoring potential adverse effects of GM feed, this would be the system detecting adverse effects and attributing them to GM when the perceived adverse health effect might be due to random error or is not actually outside of the normal variation. Similarly, the risk of type II error is the probability that an abnormality is undetected in the population. In other words, this is the probability that the surveillance system produces information that does not lead to the making of a decision although the true state of the population would require it. Type II errors imply that no decisions are made despite a genuine health issue occurring. With relation to GM products, this concern of missing adverse effects and failing to act is often cited by critics, whilst consideration to mitigating Type II errors through surveillance evaluation, should post market monitoring be enforced, would undoubtedly be of interest.

### 3.2.3. Consistency and Integration of surveillance systems.

The use of surveillance data to contribute towards rigorous scientific evidence poses many challenges. This is the case in the context of animal health diseases as well as potential adverse effects of GM feed. Surveillance systems currently in place are largely specific to a single disease and difficult to adapt to general conditions (10). Furthermore reporting is variable between countries and when surveillance is implemented for a specific condition, the usefulness of the data when applied to a different syndrome or condition needs careful consideration. Performing unnecessary surveillance is a concern particularly when resources are limited and is of interest when considering post market monitoring when there is little evidence of risk. The cost of implementing surveillance may be unjustifiable if the evidence can be obtained more accurately through other scientific

investigation methods. Data sharing between countries remains a challenge, and with such a widespread usage such as GM feed, the strength of evidence required to establish any minor unknown effects attributable to GM feed could be greatly enhanced through multi-country reporting.

### *3.3. GM feed background*

At present, decisions about the long term safety of genetically modified plants are made based on the results of risk assessments which consider a weight of evidence consisting of a broad range of data, which may or may not include controlled animal studies. The information in surveillance systems has the potential to be analysed to contribute to the longer-term monitoring of the effects of GM plants fed to animals.

To date, there are some attempted surveillance initiatives which are conducted to satisfy the requirements of post-market monitoring as and where it is recommended, such as for monitoring for possible environmental impacts of the cultivation of insect-resistant MON810 maize and for actual consumption of soybean oil with modified fat composition from imported GM soybeans used as food (29). The objective of case-specific monitoring, is to identify the possible health effects which have been recognised in the pre-market safety assessment. Contrary to general surveillance of GMOs released into the environment, case-specific monitoring is not mandatory at this stage, but decided based on the outcomes of the pre-market safety assessment.

If implemented, it is speculated that surveillance for GM feed adverse effects in animals will likely follow a similar principle to that adopted for the plant's environmental impact monitoring (13). This involves two distinct types of post-market activities which are similar in rationale to the system which is undertaken in the environmental risk assessment of GM crops (under Directive 2001/18/EC

(30)), namely: Case- Specific Monitoring and General Surveillance. The terminology is not consistent with that commonly used in Animal Health Surveillance.

### 3.3.1. General surveillance

Reports of presumed adverse effects are recorded passively after notification from farmers and other stakeholders. Regular data gathering using targeted questionnaires may be conducted depending on the animal consumption of types of GM crops. General surveillance is compulsory and a plan must be in place for all new GMOs. It is targeted to specifically identify unknown conditions. This usually entails the use of farmers' questionnaires to assess farmer's perception of symptoms or adverse effects which may have been noticed since the introduction of a new GM crop. The difficulty with such methods is that a change or symptom noted after the introduction of the novel GMO does not in itself imply the effect was due to the latter. Feedback is also solicited from feed processors and handlers if they have become aware of adverse effects. The case definition for adverse effects is non-specific, which increases the likelihood of reporting, however it reduces the probability that the effects seen may be attributable to the GM product (*i.e.* reduces the specificity of the system). An example of this system working successfully includes the farmer reporting an increase in production following the introduction of insect-resistant maize. Presumably, this is related to the decreased mycotoxin contamination of this maize. This monitoring is performed to satisfy the requirements posted by Directive 2001/18/EC on the deliberate release of GMOs into the environment (30).

### 3.3.2. Case-specific monitoring

Whilst no practical example exists as yet of case-specific monitoring of GM-feed impacts on livestock health as this has so far not been requested, this type of monitoring has been recommended for selected GM strains such as vegetable oils from GM soybean primarily used as ingredients for human food. The rationale for this stems from the need for verification of limited pre-market risk



assessments with regard to feed intake measurements. This type of monitoring has hereto not been recommended for the surveillance of animal feed. Other activities at a national level include regular sampling of food products, the objective of which is to ascertain if labelling is reliable. It may be possible to combine case-specific monitoring with future DNA analysis in the unlikely event of a large unknown adverse effect occurring.

Typically, animal health surveillance systems follow a specific approach identifying the target population and the disease of interest with a precise case definition. Tests with known sensitivities and specificities to confirm a 'true' case are standard. These are not available for any known GM feed adverse effect. As such the level of detailed planning yielding high confidence in the results for surveillance of GM feed adverse effects is not possible. When planning animal health surveillance activities, the first role is to specify the outcome and purpose for such surveillance activities. The definitions currently used by either general surveillance or case specific monitoring are broader than that used in conventional health surveillance. As such, specific recommendations at this time maybe premature; however, consideration to the options available is outlined in this paper.

#### *3.4. Applicability of health surveillance to specific scenarios*

Within a parallel activity of the MARLON project, the possibility to measure health indicators linked to various hypothetical scenarios was explored, such as potential allergenicity of GM feeds in livestock, horizontal gene transfer, and positive nutritional impacts of nutritionally improved feeds or reduced toxicity given reduced levels of natural toxins contaminating the crop (31). Also, the scenario of unknown adverse effects was considered. For this review, we considered how the various types of surveillance system could contribute to the detection of such impacts, if they were to occur, and what their strengths and weaknesses were as summarized in Table 1. What can be

inferred from this table is that all systems do have their strengths and weaknesses, which need to be considered when designing a post-market monitoring system.

<<<< INSERT TABLE 1 AROUND HERE >>>>

## 4. Review of health parameters potentially amenable to monitoring

### 4.1. Database search

#### 4.1.1. Database

A database was constructed to compile scientific research which compares livestock' productivity and health parameters of animals fed with GM feeds with animals fed with non-GM feed in a controlled manner. The studies selected for inclusion into the database had to cover health parameters (for example, clinical pathology such as serum biochemistry and haematology) beyond merely productivity and performance of livestock animals being fed GM crop-derived feeds, which has been the topic of numerous publications. As the outcomes of the literature investigations of De Vos and Swanenburg (32) showed, the number of papers providing such additional health parameters for production animals is more limited, *i.e.* 27 articles in the period 2006-2016. The aim of this exercise was to provide a repository of evidence and compile all scientific research pertaining to potential health effects beyond performance (*e.g.* haematology) in production animals related to consumption of GM varieties of cotton, potato, rice, soya and maize. The IPA Feed database provides the repository of published literature relating to genetically modified feed effects in production animals (33).

The search of original research articles was performed using Scopus (34), a large abstract and citation database of peer-reviewed literature using the following search string: TITLE-ABS-KEY ( "genetically modified" OR "genetically engineered" OR biotech\* OR transgenic ) AND TITLE-ABS-KEY ( cattle OR dairy OR cow\* OR goat OR rabbit OR farm\* OR fish OR poultry OR chicken OR hen OR pig\* OR sheep OR calf ) AND TITLE-ABS-KEY ( fed OR feeding OR consumption OR diet ) AND TITLE-ABS-KEY ( maize OR corn OR rice OR soybean OR soya OR rapeseed OR potato ).

In order to be included in the database, a study needed to fulfil each of the following criteria:

- Relevant populations: Cattle (bovine), pig, sheep, goat, poultry and fish;
- Relevant exposure: Feed containing, consisting or produced from GMOs;
- Relevant comparator: Feed containing the non-genetically modified conventional counterpart of the respective GMOs;
- Relevant outcomes: Measurements of any growth, health and productivity parameters.

One reviewer performed the selection of the studies which fulfilled the inclusion criteria at three stages. At first, articles were selected based on their title to remove clearly irrelevant studies from the overall search results, this was followed by a second stage in which the inclusion criteria were applied to the abstracts of the articles. In the third stage, the full-text articles were reviewed for the presence of each of the elements needed for their inclusion.

Details about the performed experiments were extracted from the "Materials and Methods" section of the papers, while the measurements of growth, health and productivity parameters were extracted from the tables and graphs as provided by the authors into a standardized data extraction sheet.

#### 4.1.2. Database search and inclusion criteria

A database search was performed systematically by species followed by each transformation event of GM feed. Studies were identified for inclusion if they showed a statistically significant change in one or more health or product quality-related parameters. A critical assessment of this literature was subsequently performed identifying all studies with robust methods and statistically significant results and extracting these to compile candidate parameters for evaluation. Evaluation of coherence between authors was conducted to establish if outcomes could be included in a meta-analysis. All outcomes for which there was a statistically significant effect were analysed for their suitability as outcomes for monitoring in surveillance systems. Hypothesised adverse syndromes were subsequently used to consider the strengths and weaknesses for their potential for detection in various different surveillance systems.

Whilst statistically significant effects were at the focus of this investigation, it has to be emphasized that there is a distinction between statistical significance and biological/toxicological relevance. For example, the changes observed may still be due to random error, *i.e.* the chance that the observed outcome is not a true reflection of the study population because statistical significance at  $P < 0.05$  can be due to random chance in 1/20 cases. The magnitude of any change should also be evaluated. For many of the parameters, the cut-off is not a binary variable and as such statistically significant results, even of small effect size should be treated conservatively for biological significance. It should be noted that even when the differences observed in the study reflect true differences in the study population statistical significance does not imply biological relevance or indeed causation and that whether an identified association is likely to reflect cause-effect relationship should be carefully considered in light of a number of criteria such as the strength of association, the existence of a dose response relationship, temporality, biological mechanism, repeatability, etc.. This consideration appears particularly relevant since many of these studies evaluate multiple biochemical markers increasing the probability of identifying an association as statistically significant by chance (35).

Whilst our study focused on the isolated parameters showing statistically significant differences in the various studies, there was no contradiction among the authors of the selected publications regarding the lack of biological significance of these findings, *i.e.* there were no parameters which fulfilled sufficient criteria to infer that consumption of GM feed was a risk factor for their outcome.

#### ***4.2. Identification of candidate health and production parameters.***

Besides the identification of health and production parameters for which statistically significant changes were reported, the authors explored whether there was any consistency in such effects reported for GM feed consumption and whether the design of the studies would allow for a quantitative meta-analysis of the results. Whilst there are numerous studies ( $n = 24$ ) which identified one or more statistically significant change in parameters, there is no GM crop event for which multiple studies have been conducted in the same animal species investigating health parameters beyond solely performance. As such, it is not feasible at this time to conduct a meta-analysis to establish aggregated evidence.

##### **4.2.1. Poultry**

Significant changes accrue in a study using lysine maize, a GM variety designed to improve nutrition as an alternative to direct addition of supplemental lysine to poultry diets. In this study, an increase in the rate of weight gain and feed efficiency of the birds fed with lysine maize compared to the group fed with non-GM without supplemental lysine was observed (36). Changes in the oxidation reduction potential of the breast muscles as well as in the values of thiobarbituric acid reactive substances were observed when broilers were fed with insect resistant maize and glyphosate tolerant soybean meal (37). Researchers also observed 3.7% decrease of the daily feed intake in male broilers fed with herbicide tolerant soybean (38). A tendency for higher egg weights was observed in Bt-maize-fed laying hens (39). There was a boost in the immunity against a protozoan

disease after feeding chickens with recombinant antigen expressing GM potatoes (40). Over 50% of the differences observed in these studies appear to be beneficial; as such, there is insufficient evidence to substantiate targeted surveillance in poultry.

#### **4.2.2. Fish**

In fish, the evidence appears to vary within the different studies. For herbicide tolerant soybean, there is an observed increase in feed consumption and final weight in catfish (41). A feeding study with Atlantic salmon reports minor differences in the thermal growth coefficient for the group on a diet containing insect resistant GM maize, in the plasma triacylglycerol values for herbicide tolerant GM soy diet and in hepatosomatic index for the both diets (42). Slightly lower feed intake, growth rate and final body weights were observed in the same species for the group fed with insect resistant GM maize compared with the group fed with its near-isogenic parental line (43). In an analogous study, changes in the nutrient metabolism, indicated by reduced whole-body lipid content and lipid retention efficiency, were reported (44).

#### **4.2.3. Porcine**

There is no or little evidence of adverse or beneficial effects in feeding studies involving pigs since none of the reviewed publications have reported any significant differences in the health parameters after consumption of GM containing feeds.

#### **4.2.4. Bovine**

In cattle, statistically significant but inconsistent differences (occurring once during two lactation periods) have been reported. They include a decrease in body condition score and back fat thickness, an increase in milk protein, milk urea concentration (45), and, in a different study and in contradiction to the previous one, an increase in body condition score (46). A decrease in serum blood urea nitrogen and glutamic-pyruvic acid transaminase has also been observed in a different study (47). These findings do not appear to be repeatable whilst the maize varieties fed in each one

contained different genes and originated from different transformation events (MON810, DAS-59122-7 & CBH 351).

#### 4.2.5. Multiple species

There has also been a comparison of health and performance in pigs, cows, goats and poultry before and after the large-scale introduction of GM crops into the feed supply chain over a 30 year period (48). The authors measured and reported parameters which were similar and hence not indicative of an adverse impact due to GM. These differences between studies of adverse effects possess significant challenges to identifying syndromes of interest to monitor or areas of risk which would be required in constructing surveillance programs. The variety and lack of repeatability in observed effects does not provide strong evidence for use of these parameters in animal health surveillance systems. This aligns also with our findings in the subsections above, namely that whereas some variables which showed statistically significant differences between animals fed GMO's and animals not fed GMOs.

## 5. Discussion

Monitoring in the face of uncertainty may allow for early detection of plausible adverse effects; which, could then be investigated to ascertain if GM feed were a possible cause. Considering the shortage of evidence for adverse animal health and production issues related to GM feed ingredients, however, identifying specific variables to record and test in a proposed surveillance system is challenging. Possible monitoring could consist of unexpected or expected animal health or production syndromes (*e.g.* through post-mortem inspections, (22, 23, 49)).

Unexpected events would be best evaluated via an active reporting system. Mönkemeyer and Schmidt (12) have evaluated using the sentinel surveillance at farm level for detection of health and production outcomes, which cannot be foreseen with some success unless it is ensured that attributes such as representativeness, coverage, awareness, and sensitivity are addressed.

It may be possible to use existing animal health surveillance and production data to act as an early warning system for potential adverse events. Large-scale data collection is undertaken (10), which would likely provide indications should change related to GM feed consumption occur. The interpretation of this data to inform decisions relating to GM feed, must consider the original purpose for which data were gathered and the target which the surveillance system the designed to evaluate. Possible objectives could be early warning or detection of unknown risks or estimation of prevalence, or evaluation of trends of known low-impact risks. However, these two objectives require a completely different surveillance design and implementation.

A possible comparison between organic and non-organic farming was considered but this was discounted due to concerns that any changes detected would be associated strongly with the farming system, not necessarily the exposure of interest (27). A similar reasoning would also apply to



a comparison of different member states, for which it is more likely that any differences observed between member states would be related to differing farming practices and not attributable to the consumption of GM feed. Though unconfirmed and not attributable to GM feed, the changes in health parameters observed in the feeding studies within the IPAFEED database are often proportionally small in the degree of change. Detection of such identified changes through a means such as surveillance would require investment on the scale as to make it unfeasible. Due to these challenges, it could be considered that the strength of evidence which would be provided through strengthening the current surveillance systems specifically for animal health adverse effects to GM feed would be weak. Robust cost-effectiveness analysis in the face of so much uncertainty is not feasible but it is reasonable to suppose that the monetary cost per case detectable would be high.

If post market monitoring of hypothetical effects was deemed necessary then alternative methods such as real world experimental controlled longitudinal studies would be more appropriate. However, *unknown* adverse effects could be considered in terms of sentinel surveillance as an early warning system. This is perhaps the most useful possible application of surveillance in relation to GM feed.

Data generated by surveillance systems are most of the time imperfect. The characterization of the epidemiological status of the population produced through the analysis and interpretation of such data nonetheless informs decisions. Combination of tests in either series or parallel is frequently used to increase either the sensitivity and, or the specificity of the surveillance system. As such the combination of detection methods could be used to improve sensitivity of detection of GM feed adverse effects (50). As the probability of detecting abnormalities through conventional animal health surveillance systems is low given the lack of data to support observed health impacts in livestock studies performed so far, this combination approach may help to improve sensitivity of

detection. For example detection of a similar syndrome through multiple countries or surveillance methodologies may indicate the need for further investigation.

However, for unknown adverse effects, sentinel surveillance could be considered as an early warning system. In these cases, further investigation would entail closer scrutiny, which could include observational studies assessing the relationship between the feed and health outcomes, or a controlled longer-term follow up study. This sentinel surveillance is perhaps the most useful possible application of routinely collected animal health data for post market monitoring of GM feed adverse effects. A simulation tool has been developed in the frame of the MARLON project to help design such surveillance systems. The approach consist in estimating, through probabilistic modelling, the likelihood of picking up a postulated effect based on frequency of occurrence, likelihood of clinical symptoms and likelihood of reporting by farmers, veterinarians and laboratories.

## 6. Conclusions

Several reasons make the design and implementation of animal health surveillance challenging in the context of GM, most notably the lack of evidence for known adverse effects and lack of a specific test to confirm these effects are related to GM feed. The current terminology used in GM feed post market monitoring is also not consistent with terminology frequently used in animal health surveillance.

There currently exists large scale monitoring systems in place that gather data and evaluate health and production outcomes. It is likely that if there were a major deviation in livestock health and production as a result of GM or any other exposure it would be picked up by the existing systems.

Evidence for adverse effects is a pre-requisite for case-specific post-market monitoring and literature describing such adverse effects needs to be repeatable. Investment in robustly designed pre-market health studies, if the latter are deemed necessary, may provide the strength of evidence required to inform decisions in this area of post-market monitoring. Given the lack of published evidence for adverse effects on livestock related to GM-feed consumption, it is currently not possible to target a surveillance system specifically at such effects.

For adverse effects of unknown cause observed through general surveillance of commonly measured generic health parameters, there are difficulties with both a test to associate the adverse effect to a particular factor, such as a specific GM product consumed by the animal, and also to quantify the exposure of the animal or farm to that particular GM product (50). Surveillance may be a useful alert of potential issues that may or may not be related to GM feed. Follow-up to confirm the true cause of such changes, such as through controlled studies in livestock animals, would therefore be needed

in order to confirm such attribution. The possibility of failing to detect an adverse condition which was present and detecting adverse conditions which are not truly present should be considered for any post market monitoring system for GM feed. This could be done, for example, with the use of probabilistic Monte-Carlo simulation using the approach adopted within the MARLON project. By exploring whether ongoing animal health and production surveillance could be expected to detect a postulated effect on livestock health, such an approach will support prospective applicants for GM products with the design of post-market monitoring programs that will yield meaningful outcomes, if required to do so as part of regulatory decisions for market approval under EU legislation. As explained in the introduction, post-market monitoring of a specific GM feed can be optionally required by decision makers for specific health impacts identified during the pre-market assessment. The cost-effectiveness of any surveillance approach to monitor for adverse effect of GM feed is likely to be low with a high cost per animal detected. Further work in this area may therefore be appropriate to compare its cost-efficiency with that of more controlled studies so as to ensure that the approach chosen will be proportionate to a potential health impact involved.

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## 8. References

1. James C. ISAAA Brief 52-2016: Executive Summary Ithaca NY, USA: International Service for the Acquisition of Agri-biotech Applications; 2017 [Available from: <http://www.isaaa.org/resources/publications/briefs/52/executivesummary/default.asp>.
2. Codex Alimentarius. Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants. Rome, Italy: Codex alimentarius, Joint FAO/WHO Food Standards Program, Food and Agriculture Organization; 2008.
3. EU. Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. Official Journal of the European Communities. 2003(L268):1-23.
4. Windels CE. Economic and social impacts of fusarium head blight: changing farms and rural communities in the northern great plains. *Phytopathology*. 2000;90(1):17-21.
5. FSANZ. Safety Assessments of GM Foods Canberra, Australia & Wellington, New Zealand: Food Standards Australia New Zealand; 2016 [Available from: <http://www.foodstandards.gov.au/consumer/gmfood/safety/Pages/default.aspx>.
6. FDA. CFSAN Adverse Event Reporting System (CAERS) Silver Spring MD, USA: U.S. Food and Drug Administration; 2017 [Available from: [https://www.fda.gov/Food/ComplianceEnforcement/ucm494015.htm?source=govdelivery&utm\\_medium=email&utm\\_source=govdelivery](https://www.fda.gov/Food/ComplianceEnforcement/ucm494015.htm?source=govdelivery&utm_medium=email&utm_source=govdelivery).
7. MARLON. About MARLON Wageningen, Netherlands: RIKILT Wageningen University and Research; 2015 [Available from: <http://marlon-project.eu/about.html>].
8. Salman M. Surveillance and monitoring systems for animal health programs and disease surveys. *Animal disease surveillance and survey systems: methods and applications*. 2003:3-14.
9. Halliday J, Daborn C, Auty H, Mtema Z, Lembo T, Bronsvoort BMd, et al. Bringing together emerging and endemic zoonoses surveillance: shared challenges and a common solution. *Philosophical Transactions of the Royal Society B-Biological Sciences*. 2012;367(1604):2872-80.
10. Bisdorff B, Schauer B, Taylor N, Rodriguez-Prieto V, Comin A, Brouwer A, et al. Active animal health surveillance in European Union Member States: gaps and opportunities. *Epidemiology and Infection*. 2017;145(4):802-17.
11. Bisdorff B, Schauer B, Taylor N, Rodriguez-Prieto V, Comin A, Brouwer A, et al. Active animal health surveillance in European Union Member States: gaps and opportunities. *Epidemiol Infect*. 2017;145(4):802-17.
12. Mönkemeyer W, Schmidt KCE. Use of farmer/ producer associations/ federations to form sentinel surveillance networks for adverse events in primary production. 2014;11(2):543E-n/a.
13. Bartsch D, Bigler F, Castanera P, Gathmann A, Gielkens M, Hartley S, et al. Concepts for general surveillance of genetically modified (GM) plants: the EFSA position. *Journal für Verbraucherschutz und Lebensmittelsicherheit*. 2006;1(1):15-20.
14. Triple S. Guidelines for Designing and Implementing a Syndromic Surveillance System. Saint-Maurice, France: Triple S; 2013.
15. Medina S, Sala-Soler M, Cooper D, Kanieff M, Schonemann CC, Dupuy C, et al. Guidelines to Implement or Improve Syndromic Surveillance Systems. *Online Journal of Public Health Informatics*. 2014;6(1):e145.
16. Rodriguez-Prieto V, Vicente-Rubiano M, Sanchez-Matamoros A, Rubio-Guerri C, Melero M, Martinez-Lopez B, et al. Systematic review of surveillance systems and methods for early detection of exotic, new and re-emerging diseases in animal populations. *Epidemiology and Infection*. 2015;143(10):2018-42.
17. RiskSur. Best Practices in Design, Implementation and Evaluation of Animal Health Surveillance Systems. London, UK: RiskSur Project; 2015.
18. Gibbons CL, Mangan M-JJ, Plass D, Havelaar AH, Brooke RJ, Kramarz P, et al. Measuring underreporting and under-ascertainment in infectious disease datasets: a

- comparison of methods. *BMC Public Health*. 2014;14(1):147.
19. Cameron A. *Manual of Basic Animal Disease Surveillance*. Nairobi, Kenya; 2012.
  20. Touyz LZG. Genetically modified foods, cancer, and diet: myths and reality. *Current Oncology*. 2013;20(2):e59-e61.
  21. EU. Regulation (EU) 2016/429 of the European Parliament and of the Council of 9 March 2016 on transmissible animal diseases and amending and repealing certain acts in the area of animal health (Animal Health Law). *Official Journal of the European Union*. 2016(L84):1-208.
  22. Drewe JA, Hoinville LJ, Cook AJ, Floyd T, Gunn G, Stark KD. SERVAL: a new framework for the evaluation of animal health surveillance. *Transbound Emerg Dis*. 2015;62(1):33-45.
  23. Hoinville LJ, Alban L, Drewe JA, Gibbens JC, Gustafson L, Hasler B, et al. Proposed terms and concepts for describing and evaluating animal-health surveillance systems. *Preventive Veterinary Medicine*. 2013;112(1/2):1-12.
  24. OIE. *Terrestrial Animal Health Code (2017)* Paris, France: World Organisation for Animal Health; 2017 [Available from: <http://www.oie.int/international-standard-setting/terrestrial-code/access-online/>].
  25. OIE. *Aquatic Animal Health Code (2017)* Paris, France: World Organisation for Animal Health; 2017 [Available from: <http://www.oie.int/international-standard-setting/aquatic-code/access-online/>].
  26. Meyer-Gerbault H, Batho HL, Grozdanova D. The dependence of the effective border controls on appropriate resources deployment and enhanced international cooperation. 24th Conference of the OIE Regional Commission for Europe; Astana, Kazakhstan. Paris, France: World Organisation for Animal Health; 2010. p. 29-60.
  27. Kleter G, McFarland S, Bach A, Bernabucci U, Bikker P, Busani L, et al. Surveying selected European feed and livestock production chains for features enabling the case-specific post-market monitoring of livestock for intake and potential health impacts of animal feeds derived from genetically modified crops. *Food and Chemical Toxicology*. 2018;In press:doi: 10.1016/j.fct.2017.10.004.
  28. Halliday JE, Meredith AL, Knobel DL, Shaw DJ, de C Bronsvort BM, Cleaveland S. A framework for evaluating animals as sentinels for infectious disease surveillance. *Journal of the Royal Society Interface*. 2007;4(16):973-84.
  29. Adas U. Strategy support for the Post-Market Monitoring (PMM) of GM plants: Review of existing PMM strategies developed for the safety assessment of human and animal health. *EFSA Supporting Publications*. 2015;12(1):739E-n/a.
  30. EU. Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. *Official Journal of the European Communities*. 2001(L106):1-39.
  31. De Santis B, Stockhofe N, Wal J-M, Weesendorp E, Lallès J-P, Van Dijk J, et al. Case studies on genetically modified organisms (GMOs): Potential risk scenarios and associated health indicators. *Food and Chemical Toxicology*. 2018;In press:doi: 10.1016/j.fct.2017.08.033.
  32. De Vos CJ, Swanenburg M. Health effects of feeding genetically modified (GM) crops to livestock animals: A review. *Food and Chemical Toxicology*. 2018;In Press:doi: 10.1016/j.fct.2017.08.031.
  33. IPAFEED. *IPAFEED - Information Platform for Animal Health and GM Feed* Sofia, Bulgaria: AgroBioInstitute; 2015 [Available from: <http://www.ipafeed.eu/2-uncategorised/1-welcome-to-ipafeed-information-platform-for-animal-health-and-gm-feed>].
  34. Scopus. *About Scopus* Amsterdam, The Netherlands: Elsevier; 2018 [Available from: <https://www.elsevier.com/solutions/scopus>].
  35. Hill AB. The environment and disease: association or causation? (Reprinted from *JRSM*, vol 58, 1965). *Journal of the Royal Society of Medicine*. 2015;108(1):32-7.
  36. Lucas DM, Taylor ML, Hartnell GF, Nemeth MA, Glenn KC, Davis SW. Broiler performance and carcass characteristics when fed diets containing lysine maize (LY038 or LY038 x MON 810), control, or conventional reference maize. *Poult Sci*. 2007;86(10):2152-61.
  37. Stadnik J, Karwowska M, Dolatowski ZJ, Swiatkiewicz S, Kwiatek K. Effect of genetically modified, insect resistant corn (MON 810) and glyphosate tolerant soybean meal (Roundup Ready) on physico-chemical properties of broilers' breast and thigh muscles. *Bulletin of the Veterinary Institute in Pulawy*. 2011;55(3):541-6.
  38. Herman RA, Dunville CM, Juberg DR, Fletcher DW, Cromwell GL. Performance of

- broiler chickens fed event DAS-40278-9 maize containing the aryloxyalkanoate dioxygenase-1 protein. *Regul Toxicol Pharmacol*. 2011;60(3):296-9.
39. Rasmussen MA, Cutler SA, Wilhelms K, Scanes C. Effects of Bt (*Bacillus thuringiensis*) corn on reproductive performance in adult laying hens. *Int J Poultry Sci*. 2007;6:169-71.
40. Ito A, Gotanda T, Himeno N, Itchoda N, Tabayashi N, Ike K, et al. Booster responses by oral vaccination with transgenic plants against chicken leucocytozoonosis. *Avian Pathology*. 2013;42(3):215-20.
41. Hammond BG, Vicini JL. The feeding value of soybeans fed to rats, chickens, catfish and dairy cattle is not altered by genetic incorporation of glyphosate tolerance. *The Journal of nutrition*. 1996;126(3):717-27.
42. Sanden M, Krogdahl Å, Bakke-Mckellep A, Buddington R, Hemre GI. Growth performance and organ development in Atlantic salmon, *Salmo salar* L. parr fed genetically modified (GM) soybean and maize. *Aquaculture Nutrition*. 2006;12(1):1-14.
43. Hemre GI, Sagstad A, Bakke-McKellep AM, Danieli A, Acierno R, Maffia M, et al. Nutritional, physiological, and histological responses in Atlantic salmon, *Salmo salar* L. fed diets with genetically modified maize. *Aquaculture Nutrition*. 2007;13(3):186-99.
44. Gu J, Krogdahl A, Sissener NH, Kortner TM, Gelencser E, Hemre GI, Bakke AM. Effects of oral Bt-maize (MON810) exposure on growth and health parameters in normal and sensitised Atlantic salmon, *Salmo salar* L. *Br. J. Nutr*. 2013;109(8):1408-1423
45. Steinke K, Guertler P, Paul V, Wiedemann S, Ertle T, Albrecht C, et al. Effects of long-term feeding of genetically modified corn (event MON810) on the performance of lactating dairy cows. *Journal of animal physiology and animal nutrition*. 2010;94(5).
46. Brouk MJ, Cvetkovic B, Rice DW, Smith BL, Hinds MA, Owens FN, Iiams C, Sauber TE. (). Performance of lactating dairy cows fed corn as whole plant silage and grain produced from genetically modified corn containing event DAS-59122-7 compared to a nontransgenic, near-isogenic control. *Journal of dairy science*. 2011;94(4): 1961-1966.
47. Yonemochi C, Ikeda T, Harada C, Kusama T, Hanazumi M. Influence of transgenic corn (CBH 351, named Starlink) on health condition of dairy cows and transfer of Cry9C protein and cry9C gene to milk, blood, liver and muscle. *Animal Science Journal*. 2003;74(2):81-8.
48. Snell C, Bernheim A, Berge J-B, Kuntz M, Pascal G, Paris A, et al. Assessment of the health impact of GM plant diets in long-term and multigenerational animal feeding trials: A literature review. *Food and Chemical Toxicology*. 2012;50(3-4):1134-48.
49. Howe KS, Hasler B, Stark KD. Economic principles for resource allocation decisions at national level to mitigate the effects of disease in farm animal populations. *Epidemiol Infect*. 2013;141(1):91-101.
50. Nadal A, De Giacomo M, Einspanier R, Kleter G, Kok E, McFarland S, et al. Exposure of livestock to GM feeds: Detectability and measurement. *Food and Chemical Toxicology*. 2018;In press:doi: 10.1016/j.fct.2017.08.032.

**Table 1: Strengths and Weaknesses of Surveillance programs for GM feed syndromes.**

Type of Surveillance Activity	Syndrome of Outcome of Interest				
	Allergenicity	Horizontal Gene Transfer / AMR	Unknown Adverse Effects	Beneficial Effects. Eg Inc growth rate.	
Passive / Enhanced Passive	<b>Strengths</b>	Cheap, infrastructure in place.	Low start up costs.	Higher reporting rate than other conditions	Low set up cost.
	<b>Weaknesses</b>	Not specific to allergenicity. Prone to type II errors.	Varies rapidly, not specific to GM feed. Poor proof of attribution.	Missclassification may be problematic. Poor proof of attribution.	Standard passive system would have a low specificity here.
Meat Inspection	<b>Strengths</b>	Add onto existing activities, It may be possible to perform DNA analysis on stomach contents.	Swabs and DNA analysis could be performed.	Slaughter line already established to evaluate for unknown and general meat hygiene issues.	Evidence of beneficial effects eg. carcass weight etc. could be evaluated.
	<b>Weaknesses</b>	No evidence gross PM will detect and lesions. DNA tests would be costly.	Difficult to show attribution, routine swabbing and DNA testing would be extremely costly.	High probability of false negatives.	Missclassification of the cause of the effect.
Active Targetted	<b>Strengths</b>	Increased specificity.	Increased specificity	Increased compliance & specificity.	Increased specificity.
	<b>Weaknesses</b>	Condition has low detection rates.	Difficult to target on as AMR requires in depth DNA analysis of feed and bacteria to show attribution.	under reporting and false positives may occur	May lead to type II error.
Sentinel Surveillance	<b>Strengths</b>	Strategic, high exposure farms could be used to reduce costs.	Strategic, high exposure farms could be used to reduce costs.	Strategic, high exposure farms could be used to reduce costs.	Strategic, high exposure farms could be used to reduce costs.
	<b>Weaknesses</b>	Poorly specific to allergenicity.	Difficult to show attribution, routine swabbing and DNA testing would be	under reporting and false positives may occur	May lead to type II error.