SESSION 5

ACHIEVING FOOD SAFETY AND SECURITY IN THE 21ST CENTURY
Biosecurity in a Global Market Place

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ABSTRACT

International travel and free trade are modern bywords and the international movement of people, animals and livestock products seen as essential for the global market place to function. Yet is this compatible with a national bio-secure environment? Governments around the world seek to manage the risks posed by infectious disease to livestock, man, the environment and related ecosystems whilst at the same time permitting free trade. Ample examples exist of these competing elements as illustrated by recent outbreaks of avian influenza, bluetongue, severe acute respiratory syndrome (SARS) and most recently in Australia, equine influenza. Whilst the recognition that some 70% of new infectious diseases in man come from animals, even those diseases that affect only animals such as foot and mouth disease, can have devastating effects on trade and economies. The word ‘biosecurity’ now encompasses most of these elements with processes being developed to identify, mitigate or eliminate these biosecurity risks, and ultimately to prevent adverse events. An added dimension to be considered recently is that of bio-terrorism. So is it time for a new global co-ordinated and collaborative approach to managing biosecurity that recognises the need to encourage not restrict, the global market place? Are there newer approaches that could encourage global trade in livestock and livestock products? One such strategy could be to consider the biosecurity risks of the commodity as opposed to the disease status of the country of origin as a more effective approach for the future.

Key words: trade, infectious diseases, biosecurity, risk assessment, health status, commodity-based, international standards.

THE GLOBAL MARKET PLACE FOR LIVESTOCK AND LIVESTOCK PRODUCTS

In 1999 it was recognised that a global livestock revolution was underway characterised by a doubling in demand for livestock products over the next 15 years (Delgado et al., 1999). Driven by urbanisation, an increase in available incomes and a move away from a cereal- to a livestock-based diet, it was foreseen that this demand-driven process would provide a major opportunity for those in the livestock sector. Whilst not a global trend, it was clear that in both Asia and Latin America this revolution had the potential to seriously revitalise rural communities and for many provide a way out of subsistence farming. Now ten years on, every indication continues to support this basic premise (Dijkman et al., 2008). However, more recently there has been a shift of production away from the temperate and dryer areas, to the warmer and more humid areas but with an associated increase in livestock disease risks for producers in such regions. In response to these and other pressures, there has been a move from local multi-purpose activities to a more market orientated integrated process-driven production approach. Significantly, this has increased pressure on communal resources such as grazing areas and water.

As the livestock revolution has unfolded, in some regions there has been a focus on large-scale industrial type production systems with a major concentration on monogastric species (pigs and poultry) (Naylor et al., 2005). There are, however, large regional differences e.g. Brazil has emerged as the major global poultry and pig producer with China and Japan as major importers whilst sadly, the situation in Africa has remained static. Critically these changes have been associated with significant threats to both the environment and human health. The overall trend has continued to be associated with significant shifts of production from developed to developing countries (Figure 1).

FOOD SECURITY

There has been a growing focus on global food security since the Millennium Development Goals were set, and whilst still targeting developing countries the global nature of this challenge has become increasingly clear. The matter has now become significantly more acute with the new awareness of the impact of climate change on primary food production and the vital role played by water availability in the process. For livestock, the negative impact of methane production and the associated carbon trading issues have somewhat tempered producer opportunities. A major factor leading to volatility has been the shift from developed to developing countries.

Figure 1. Shifting production of livestock products from developed to developing countries.

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in the financial viability of supply chains has been the availability and price of grain. Considered within the concept of the three ‘Fs’ (feed, fuel and food), the influence of grain has been significant in the past ten years. Effects such as the availability and price of oil, and the use of grain as an alternative fuel source have had profound effects (Steinfeld, 2003). Yet the financial viability of livestock as a key food source depends intrinsically on the price of grain. The longer term implications of these fluctuations on livestock production profitability remain unclear.

DO INFECTIOUS DISEASES MATTER?

In the late 1980’s infectious disease both of man and animals were considered threats of the past. The range of effective vaccines and therapeutics had persuaded many that health risks of the future would be focused on nutritional diseases and those associated with longevity. This paradigm, however, changed somewhat dramatically with the advent of mad cow disease and then subsequently, to name but a few, SARS, avian influenza, foot and mouth disease and most profoundly, AIDS (Woolhouse et al., 2005). By the turn of the century it was clear that not only were new and emerging diseases having a profound impact e.g. SARS, henipavirus infections, but that many diseases thought to be under control were re-gaining importance e.g. tuberculosis, malaria.

An example of the speed of spread can be seen with the introduction of bluetongue into Europe. The disease had been confined to one or two islands in the Mediterranean area but this changed dramatically in 2002 with an introduction into Holland and the subsequent spread throughout Europe.

Importantly, the cost of disease relates not only to the effect on the individual animal and producer, but the knock-on effects to other industries and related areas. The 2001 foot and mouth disease outbreak in Europe, whilst serious enough for livestock producers, had a huge impact on the tourist industry in the UK, whilst for the global SARS pandemic, the biggest economic impact was on the airline industry (Figure 2).

Overall, the incidence of infectious disease continues to grow globally, with known infectious diseases gaining further ground e.g. rabies, tuberculosis, salmonella, Rift Valley fever; and with new diseases continuing to arise e.g. acquired immunodeficiency syndrome (AIDS), bovine spongiform encephalopathy (BSE), Hendra virus infections, Nipah virus infections, SARS, and Ebola Reston virus. Significantly 75% of new diseases affecting man now originate in animals (Jones et al., 2008). Gradually the new concept of ‘one health’ has emerged within the framework built around the ‘biosecurity’ approach. Biosecurity is essentially about the risks associated with infectious disease and the related causative pathogens and has now been extended by some to include all invasive species and their impact on animals, man and the environment (Figure 3).

THE CONCEPT OF RISK

Managing the threats from infectious disease requires an understanding of the risks involved and an underlying perception that there are no certainties (Wooldridge et al., 2006). The threats are unpredictable and the associated risks need to be managed within this framework. Risk is determined through an analysis of likelihood and consequence with a crucial underpinning i.e. understanding that zero — risk does not exist. In risk management it is necessary at the outset to undertake a hazard identification followed by a detailed risk assessment before considering risk mitigation strategies and importantly a process of risk communication. A useful approach is to consider the twin elements of likelihood and consequence within the framework of a ‘Bow Tie’ structure (Figure 4).

A careful review would indicate that the biggest biosecurity risk posed by invasive species comes from those infectious agents that can evolve and adapt both to their current hosts and through host switching. In this context, the virus is ideally suited and therefore

Figure 2. Economic impacts of selected emerging and re-emerging infectious diseases.
The OIE was established in 1924 to manage the risks of animal disease brought about by trade in livestock (e.g. rinderpest and foot and mouth disease). At this time it was recognised that the main risks were associated with the movement of live animals and therefore there was a clear focus on establishing the disease status of the country of origin of the animals being imported. This resulted in trade essentially taking place with countries of similar disease status. Although increasingly this provided a real impact. Developing countries are placed at a serious disadvantage and unfortunately many of these countries which have agriculturally-based economies find themselves unable to trade effectively in their primary commodity. The starkest examples exist in Africa, where resources for establishing and maintaining veterinary services remain limited, international trade in livestock and livestock products is minimal and these countries remain within the ‘poverty trap’. Is there an alternative?

Commodity Versus Product

In the past few years, the OIE has begun to accept the principle of commodity-based trading (CBT) and uses this as a guide for bilateral trade agreements (see www.oie.int). It is important to distinguish this from the extensive work undertaken by the FAO/WHO Codex Alimentarius Commission which deals with setting standards for products, albeit confined exclusively to issues of human food safety. There are regrettable overlaps and gaps in the standards established for commodities (OIE) and products (Codex Alimentarius). Harmonising these standards could do a great deal but would still require drastic modification of current certification and auditing procedures to have a real impact.

Building on this Paradigm

Can we therefore build on this alternative approach and trade in the livestock commodity or processed product and not in the live animal — and base the biosecurity risk assessments on the commodity or product rather than on the disease status of the country (Perry et al. 2005)? For subsistence farmers in developing countries participation in the ‘livestock revolution’ is essential to create a pathway out of poverty.

Support for driving this change would need to focus on establishing processing plants and operations in developing countries and to undertake production in ways that address any biosecurity risks in the final exported product. The national disease status thereby becomes irrelevant and risk is assessed from the perspective of the commodity and not the animal. This approach would involve further investment in infrastructure to create the necessary post-farm gate processing capability but could have real advantages for the many developing countries currently excluded from the international livestock market place because of their current disease status. In creating this processing infrastructure, ways would need to be found to fully engage livestock producers in the process, through, for example, whole of chain co-operatives. In this way the benefits would feed all the way down the production chain and not just accrue post-farm gate.

World Organisation for Animal Health (OIE)

The OIE was established in 1924 to address issues are now being addressed by OIE and it works in close co-operation with the World Health Organization in addressing issues around zoonotic diseases. However, despite all these changes, for most developing countries, the fundamental basis for trade is still as it was in 1924 i.e. the health status of the ‘national herd’.

The OIE approach is not the same today (OIE, 2008) and the OIE’s Terrestrial Animal Health Code (TAHC) now permits the use of zonation and compartalisation to assist trade. There is now a more sophisticated risk framework, which clarifies the processes for defining the disease status of a country, detailed guidelines on the laboratory tests to be used, and processes for assessing the quality of national veterinary services and their ability to correctly determine a national disease status. Although not directly a requirement, animal welfare issues are now being addressed by OIE and it works in close co-operation with the World Health Organization in addressing issues through.

But viewed from some perspectives, this is not a ‘level’ playing field. Developing countries are placed at a serious disadvantage and unfortunately many of these countries which have agriculturally-based economies find themselves unable to trade effectively in their primary commodity. The starkest examples exist in Africa, where resources for establishing and maintaining veterinary services remain limited, international trade in livestock and livestock products is minimal and these countries remain within the ‘poverty trap’. Is there an alternative?

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Successes in this area have already been achieved, as for example in Kenya and Ethiopia (Perry et al., 2005) but international bodies such as the World Bank need to drive the process further to ensure the necessary investments are available to establish the needed infrastructures including processing plants. Of course, none of this precludes the need to continually improve the disease status of these countries, to improve their veterinary services and reduce the risks from disease on their livestock, people and ecosystems. Indeed the more these issues are addressed at the level of the farm the less will be the need to manage the risks at the processing end.

CONCLUSIONS

Global trade in livestock and livestock products continues to increase and is now clearly demand- rather than supply-driven. Ensuring a food secure environment will therefore in part only be achieved through meeting this demand. Biosecurity and particularly the risks from infectious diseases in live animals limit our ability to meet this demand at the global level. Investments in processing capacity at or near livestock production areas and importantly, prior to export from a country, could significantly reduce biosecurity risks. This has the potential to provide substantial benefits to those countries currently excluded from the opportunities provided by the livestock revolution because of their poor national disease status. Is this the way for livestock producers in developing countries to trade their way out of the poverty trap?

REFERENCES


Quality Comparison between Gamma-Irradiated and E-beam Irradiated Pork Patties


ABSTRACT
This study compared the effects of gamma and electron beam (e-beam) irradiation on the quality of pork patties. Pork patties (diameter: 100 mm, thickness: 10 mm) were vacuum-packaged and irradiated by gamma ray ($^{60}$Co with a 490 kCi source) and e-beam (2.5 MeV) at five, ten, 15, and 20 kGy at room temperature. During accelerated storage at 30°C for 10 d, determination of total bacterial populations, hardness, and sensory evaluation was conducted at appropriate sampling intervals. The results of total bacterial populations showed that the gamma-irradiated (GR) samples had lower (P < 0.05) total bacterial counts than e-beam-irradiated (EB) samples during storage at 30°C for 10 d, regardless of irradiation dose. The hardness and sensory properties such as colour, chewiness, taste, and overall acceptability of pork patties were decreased depending upon irradiation dose. GR samples had lower hardness and sensory scores than those of EB samples. In conclusion, gamma irradiation on pork patties should be useful in decreasing bacterial populations when compared with e-beam irradiation. However, further studies should be conducted to reduce the quality deterioration of GR pork patties.

Key words: pork patties, gamma irradiation, E-beam irradiation, bacterial populations, hardness, sensory properties.

INTRODUCTION
The meat processing industry has grown substantially in recent years, and the development of new processed meat products has increased because of the demand for ready-to-eat meat products and the excellent nutritional properties of the foods. However, slaughter, cutting, and processing procedures may increase the possibility of microbial contamination of foods. The studies by Taha (1999), and Woodburn and Raob (1997) showed that fresh meat and processed meat have been implicated in the transmission of foodborne pathogens such as *Escherichia coli* O157:H7, *Salmonella*, *Pseudomonas* spp., *Listeria monosytogenes*, *Staphylococcus aureus*, and *Bacillus cereus*.

One of the decontamination technologies for ensuring the microbiological safety of meat is radiation processing. In addition to spoilage bacteria, meat products may contain parasites and pathogenic bacteria, which can be inactivated by irradiation (Olson, 1998). Many researchers have also reported that gamma or electron beam (e-beam) irradiation in low doses (< 10 kGy) kills most microorganisms with no deterioration of food quality (Mohamed, 1999; Thayer et al., 1995; Youssef, 1994). Indeed, several reports have demonstrated the antimicrobial effects of radiation in meat products such as bacon, ham (Weirbicki and Heiligman, 1980), hamburgers (Dempster et al., 1985) and sausage (Kiss et al., 1990).

Meanwhile, Mitchell (1994) suggested that e-beam processing is regarded more favourably than gamma irradiation by consumers who may associate gamma processing with the nuclear industry. Thus, comparison was needed of the effects of gamma and e-beam on microbial, physicochemical and sensory properties of different foods. However, few studies have been conducted to compare the effects of gamma and e-beam irradiation on quality and reduction of bacterial populations in meat and meat products (Mitchell, 1994; Song et al., 2009; Park et al., 2010). Therefore, the objective of this study was to compare the effects of gamma irradiation and e-beam irradiation on the qualities of the pork patties as well as reduction of microbial population.

MATERIALS AND METHODS

Pork Patty Preparation and Packaging

Pork loins were purchased from three local grocery stores. Ground pork (53 g) was then mixed with various ingredients: (pork back fat: 15 g, ice water: 6 g, ginger: 1 g, onion: 8.5 g, egg white: 4.3 g, tomato ketchup: 1.6 g, isolated soy protein: 4.1 g, dried bread powder: 2.5 g, nutmeg powder: 0.05 g, NaCl: 0.65 g, flavour enhancing wine: 0.41 g, black pepper powder: 0.21 g, red colour reagent: 0.01 g, trisodium phosphate: 0.22 g, sugar: 0.85 g) as described by Lee et al. (2005), and 100 g of the meat batter was used to prepare patties (diameter: 100 mm, thickness: 10 mm) using a patty maker (Large Hamburger press, Tupperware, Inc., Orlando, FL, USA). The patties were then heated in a cooker (NUVIES–3 cooker, Menominee, MI, USA) up to 70°C of internal temperature, removed from the cooker and cooled down at room temperature (25°C). Each patty was placed in a retort pouch laminated with polyester, aluminum and polypropylene (MULTIVAC, Wolfertschwenden, Germany), followed by vacuum-packaging. The internal temperature was monitored with a thermocouple (TES–1300 thermometer, TES, TAIWAN).

Irradiation and Storage Conditions

The vacuum-packaged samples were irradiated at 0 (control), 5, 10, 15, and 20 kGy of gamma rays, while the e-beam irradiated both sides of the patties at same dose as the gamma irradiation. Gamma irradiation was conducted using a $^{60}$Co irradiator (point source AECL, IR–79, MDS Nordion International Co. Ltd., Ottawa, Ontario, Canada) in the Advanced Radiation Technology Institute, Korea Atomic Energy Research Institute (Jeoung-Eup, Korea). The source strength was 1 Team for Radiation Food Science & Biotechnology Advanced Radiation Technology Institute, Korea Atomic Energy Research Institute, Jeoung-Eup, Korea.

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approximately 300 kCi with a dose rate of 10 kGy/h. Dosimetry was applied using 5 mm diameter alanine dosimeters (Bruker Instruments, Rheinstetten, Germany). E-beam irradiation was performed with an ELV–4 Electron-Beam-Accelerator (2.5 MeV) at the EB-Tech (EB-Tech Co., Daejeon, Korea). The beam currents were 2.5, 5, 7.6, and 10.5 mA for 5, 10, 15, and 20 kGy, respectively.

After irradiation, the patties were stored in an incubator (Mir 552, Sanyo Co., Tokyo, Japan) at 30°C for 10 d.

**Total Aerobic Bacteria**

Total aerobic bacterial populations in patties were determined on days 0, 2, 5, and 10. The 10 g portions of patties were placed aseptically in sterile nylon bag (10 × 15 cm; Sunkyung Co., Ltd., Seoul, Korea) containing 90 mL of 0.1% sterile peptone water (Difco Laboratories, Detroit, MI, USA) and blended for 2 min using a Lab-blender 400 stomacher (Seward medical, London, UK). The blended sample was used to test the growth of the total aerobic bacterial populations in a plate count agar (Difco Lab., St. Louis, USA). Plates were prepared in triplicate and incubated at 37°C for 48 h, and aerobic bacterial populations on a plate were determined as colony forming units (log CFU)/g.

**Hardness and Sensory Evaluation**

The hardness of the patties was also determined on day 0 using a penetrating test by a texture analyser system (TA-XT2i, Stable Micro System, England) equipped with a probe (1.0 cm thickness). Sensory evaluation of the patties was conducted by 21 panelists who were trained according to the method described by Civille and Szczesniak (1973). Colour, chewiness, taste, off-flavour, and overall acceptance of non-irradiated, gamma and e-beam irradiated samples were evaluated using a seven-point descriptive scale where 1 = extremely disliked or extremely weak to 7 = extremely liked or extremely strong. After irradiation, patties were removed from pouches and reheated in a cooker (NUVUES–3 cooker, Menominee, MI, USA) at 130 °C for 10 min for sensory evaluation.

**Statistical Analysis**

One-way analyses of variance (ANOVA) were used to determine the effect of a combined treatment of *Kimchi* on the growth of the microorganisms and the quality properties of four groups by a Statistical Package for Social Sciences (SPSS, 10.0). Duncan’s multiple range test was used to compare the differences among the means at P < 0.05.

**RESULTS AND DISCUSSION**

**Effect of Irradiation on Bacterial Growth**

After irradiation (day zero), gamma irradiation decreased bacterial populations more than e-beam irradiation (P < 0.05), and GR samples at more than 5 kGy and EB samples at more than 10 kGy had levels

<table>
<thead>
<tr>
<th>Days</th>
<th>Gamma ray (kGy)</th>
<th>Electron beam (kGy)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3.65</td>
<td>ND 2)</td>
</tr>
<tr>
<td>2</td>
<td>6.89</td>
<td>7.91</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>6.83</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>6.61</td>
</tr>
<tr>
<td>15</td>
<td>-</td>
<td>5.51</td>
</tr>
</tbody>
</table>

1 indicates no determination of cells because of spoilage.
2 not detectable.

**Table 2. Evaluation of hardness and sensory qualities of gamma ray or electron beam irradiated pork patties after vacuum packaging.**

<table>
<thead>
<tr>
<th>Dose (kGy)</th>
<th>Hardness (g)</th>
<th>Colour</th>
<th>Chewiness</th>
<th>Taste</th>
<th>Off-flavour</th>
<th>Overall acceptance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gamma ray</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>0</td>
<td>431.76±45.35a</td>
<td>6.8±0.8a</td>
<td>6.7±0.7a</td>
<td>6.8±0.6a</td>
<td>1.2±0.1c</td>
<td>6.8±0.8a</td>
</tr>
<tr>
<td>5</td>
<td>395.67±50.32a</td>
<td>6.1±0.6a</td>
<td>6.1±0.5a</td>
<td>6.2±0.4a</td>
<td>2.2±0.2b</td>
<td>5.7±0.4a</td>
</tr>
<tr>
<td>10</td>
<td>385.06±27.59a</td>
<td>5.6±0.5ab</td>
<td>5.7±0.4ab</td>
<td>5.6±0.6a</td>
<td>2.7±0.3ab</td>
<td>5.3±0.5ab</td>
</tr>
<tr>
<td>15</td>
<td>381.43±20.32a</td>
<td>5.1±0.4b</td>
<td>5.2±0.4b</td>
<td>5.3±0.4ab</td>
<td>3.1±0.3a</td>
<td>4.6±0.2b</td>
</tr>
<tr>
<td>20</td>
<td>375.69±28.35a</td>
<td>4.7±0.5b</td>
<td>4.4±0.3b</td>
<td>4.1±0.3b</td>
<td>3.3±0.2a</td>
<td>4.2±0.4b</td>
</tr>
<tr>
<td>Electron beam</td>
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<td></td>
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<tr>
<td>0</td>
<td>431.76±45.35a</td>
<td>6.7±0.6a</td>
<td>6.9±0.6a</td>
<td>6.7±0.7a</td>
<td>2.1±0.2b</td>
<td>6.9±0.5a</td>
</tr>
<tr>
<td>5</td>
<td>424.38±36.22a</td>
<td>5.9±0.6a</td>
<td>5.8±0.6ab</td>
<td>6.5±0.3a</td>
<td>2.3±0.2ab</td>
<td>5.6±0.4b</td>
</tr>
<tr>
<td>10</td>
<td>423.21±16.62a</td>
<td>5.8±0.5ab</td>
<td>5.5±0.3b</td>
<td>5.8±0.5ab</td>
<td>2.7±0.1a</td>
<td>5.4±0.4bc</td>
</tr>
<tr>
<td>15</td>
<td>419.93±83.64a</td>
<td>5.4±0.3b</td>
<td>4.6±0.4c</td>
<td>5.4±0.6b</td>
<td>2.9±0.2a</td>
<td>4.9±0.2c</td>
</tr>
<tr>
<td>20</td>
<td>407.34±69.88a</td>
<td>5.1±0.4b</td>
<td>4.3±0.3c</td>
<td>4.3±0.4c</td>
<td>2.7±0.2a</td>
<td>4.4±0.4c</td>
</tr>
</tbody>
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* Means within the same column different letters differ significantly (P < 0.05).
below detection limit (2 log CFU/g) (Table 1). This was also found in studies by Chung et al. (2000), Song et al. (2009) and Park et al. (2010) indicating that gamma irradiation was more effective than e-beam irradiation for the destruction of P. fluorescens or total aerobic bacteria in refrigerated beef or beef patties. However, bacteria in the samples below the detection limit were recovered during accelerated storage at 30°C.

**Hardness and Sensory Evaluation**

The results of the hardness and sensory evaluation of patties are shown in Table 2. The hardness of the GR and EB samples significantly decreased (P < 0.05) depending on the irradiation doses. The hardness of the GR patties was lower than that of the EB patties. Yook et al. (2001) studied the effect of gamma irradiation on morphological properties and post-mortem metabolism in bovine M. sternormandibularis with special references to ultrastructure, shear force, pH, and ATP breakdown. This observation suggests that the bonds between myosin and actin are disrupted by irradiation and is supported by the report of Lee et al. (2000), that myosin was denatured by gamma irradiation.

Sensory properties such as colour, chewiness, taste, and overall acceptability of the GR and EB samples were decreased depending on the irradiation doses (Luchsinger et al., 1996; Park et al., 2010). The GR samples had lower sensory scores than the EB samples. These adverse changes (off-flavour) may be caused by free radicals generated from irradiation (Smith et al., 1960). However, the generation of off-flavour in irradiated meat and meat products can be reduced by various methods such as modified atmosphere packaging, reducing the temperature (freezing) prior to irradiation and addition of antioxidants (Brewer, 2009).

**CONCLUSIONS**

Gamma irradiation is more effective for inactivating microorganisms in patties than e-beam irradiation. However, gamma irradiation decreased the hardness and sensory scores of patties to a greater extent than e-beam irradiation. Therefore, combination treatments such as modified atmosphere packaging, reducing the temperature (freezing) prior to irradiation and addition of antioxidants will be necessary for quality improvement of irradiated patties.

**REFERENCES**


Current Status, Surveillance and Control of Avian Influenza in Domestic and Wild Bird Populations in Bulgaria

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ABSTRACT
This report describes the history and current status of avian influenza (AI) infection and control in Bulgaria. The country has a unique geographic position in Europe with regard to wild bird populations and their migration routes which pass through its territory. In recent years, Bulgaria did not remain free from AI. The region with the highest rate of isolation of H5N1 virus strains were the Black Sea coast and wet territories connected with the Via Pontica migration pathway in the administrative districts of Dobrich, Varna and Bourgas. Low pathogenic (LP) AI strains isolated from ducks were subtypes H3, H4, and H6 from the areas of Plovdiv, Pazardjik, St. Zagora, Yambol, Sliven and Haskovo. Raising ducks for liver production is a popular practice in south and southeast Bulgaria. From an epidemiological standpoint, controlling circulation of AI viruses among duck flocks, especially before their gathering in larger farms for fattening is a mandatory requirement of official authorities. To prevent the spread of highly pathogenic (HP) AI, surveillance of domestic poultry as well as wild birds should be strengthened in countries at risk, especially along bird migration routes. Monitoring, sampling and analysis of the viral subtypes of AI found in wild birds needs to be carried out to fully understand their role in the propagation and spread of HPAI viruses.

Key words: Avian influenza, Bulgaria, migratory routes, National Veterinary Service, wetlands, wild birds.

INTRODUCTION
Avian influenza (AI) is a highly contagious viral disease affecting domestic birds (chickens, turkeys, quails, guinea fowl, etc.), as well as pet birds and wild birds. It is a disease of varying severity but may be of great importance for animal health, with serious implications for the poultry industry and, in some cases, for human health. Influenza A viruses infecting poultry can be divided into two distinct groups on the basis of their ability to cause disease in susceptible birds: low-pathogenic AI (LPAI) and highly pathogenic AI (HPAI) (Lamb and Krug, 2001; Fouchier et al., 2005). Highly pathogenic avian influenza (HPAI) virus spreads rapidly, and may cause serious disease and high mortality in affected birds (up to 100% within 48 h). The low pathogenic avian influenza (LPAI) causes mild disease that may be undetected as some species of birds show no symptoms. Subtyping of influenza A viruses is based on antigenic differences between the two surface glycoproteins hemagglutinin (HA) and neuraminidase (NA). To date, 16 HA and 9 NA subtypes of influenza A viruses have been identified.

Wild birds play a role on the circulation of influenza A virus. They often carry LPAI viruses (Alexander, 2000; OIE, 2004; Mekushinov, 2006), and infected individuals can spread these over a wide area during their migration between breeding and wintering grounds. The mallard is one of the most abundant waterfowl in the world, and Munster et al. (2005) detected numerous influenza A virus subtypes, including the strains H5 and H7, which may sometimes be highly pathogenic in this species (Mekushinov, 2006).

Once domestic birds are infected, outbreaks caused by HPAI can be difficult to control and often have major economic impacts for poultry farmers in affected countries, since mortality rates are high and infected birds must be destroyed in order to prevent the spread of the disease. Indeed, since 1997 millions of domestic poultry died or had to be destroyed due to outbreaks of HPAI H5N1 in the countries of Southeast Asia.

AVIAN INFLUENZA IN BULGARIA
The Danube Delta forms the most extensive wetland in Europe after the Volga delta. This is one of Europe’s most important sites for breeding, passage and wintering of water birds, particularly wintering; it regularly holds more than 20 000 water birds. In winter 2005–2006, the Danube Delta area faced several outbreaks of HPAI H5N1 in both domestic and wild birds. H5N1 avian influenza was first reported in Romania in October 2005. In February 2006, the OIE confirmed a further outbreak in poultry in the Jurilovca district of Tulcea County. Several outbreaks had previously been reported in this County, although this was the first in the Jurilovca district. Birds on the infected farm and neighbouring premises were culled and movement controls on people and poultry applied.

The poultry livestock industry in Bulgaria is still not affected by the AI subtype H5N1 virus. However, in 2006 a pathogenic strain was isolated from five sick and dead swans and in the course of surveillance for AI viruses (AIVs) low pathogenic strains of types H4, H5, H6, H7 and H10 were also isolated. These findings show that the country is threatened by potential AI infection connected with wild migratory waterfowl.

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Bulgaria possesses a unique flora and fauna with some 394 wild bird species recorded in the country. Bulgaria has a special geographic position in Europe with regards to wild bird populations (Nankinov et al. 1997; Kostadinova and Gramatikov, 2000) and is the second country in Europe after Spain in terms of the diversity of its bird fauna. Although the territory represents only 1% of the European land mass, it contains 76% of the European ornitho-fauna (Nankinov et al. 1997). Many of them are migratory birds and some are included in the Red Book of endangered species. There are two major migratory routes that wild birds use to cross the territory of Bulgaria - the Via Pontica and Via Aristotel. These are used by migratory birds from Northern, Central and Eastern Europe and western Siberia.

Anatidae (ducks, geese and swans) are a group of water birds that are ecologically dependent on wetlands for at least some aspects of their annual cycle. These species use a wide range of wetlands, from the high arctic tundra, rivers and estuaries, freshwater or saline lakes, and ponds or swamps, to coastal lagoons and inter-tidal coastal areas such as mud-flats, bays and the open sea. They also utilise man-made wetlands such as rice fields and other agricultural areas. Many Anatidae populations migrate between wetlands in the northern breeding areas and southern non-breeding areas and in doing so, regularly cross the borders of two or more countries.

These migrations create possibilities for multiple contacts with other bird and animal species and enhanced circulation and spread of AIVs. It is well known that most of the influenza A subtype viruses circulate in their principal host, the wild duck. The main migratory species in Bulgaria belong to two orders, Anseriformes (ducks, geese, swans, etc.) and Charadriiformes (Arenaria interpres, Vanellus spinosus, Scolopax rusticola, Sterna fuscata, Larus crassirostris, etc.).

One of the widespread migrating species in the country is the mallard (Anas platyrhynchos), which has a breeding population of up to 6 000 birds and a wintering population of up to 148 600 birds (BirdLife International, 2004). The greatest numbers are observed in October, with numbers falling until the end of December, and increasing again in January (Munster et al., 2005; Olsen et al., 2006). This is because the autumn migration has two stages. The first one is the movement of mallards to the Mediterranean Sea and western Asia, mainly during October and November, while the second comprises mallards that come for wintering in Bulgaria at the end of December and beginning of January. During the spring migration, which takes place in February until mid-March, mallards go back to northeastern Russia and Siberia (Munster et al., 2005; Olsen et al., 2006). Wintering of birds from Central Europe in Bulgaria is considered an exception (Munster et al., 2005).

High and Low Pathogenic Strains Isolated

The prevalence of infection with AI has a seasonal character, being greatest in late autumn and winter in the Northern hemisphere, when the birds come back from the regions where they lay their eggs and care for the young, namely the Arctic zone where there is a very dense presence of different bird species and nests and oral/faecal contamination is massive. Clinical symptoms of the disease appear in some of the resting stops during the migration e.g. an H5N1 epizootic in Romania in 2005 and cases of infected swans and wild ducks in the period after January 25, 2006 in Bulgaria. The severe cold of –25 °C was thought to be the cause of dissemination in all directions and to neighbouring Romania of subtype H5N1 by sick and infected swans.

The regions in Bulgaria within the last three years where isolates of highly pathogenic viruses of the H5N1 subtype and the low pathogenic viruses of H4, H6, H7 and H10 have been found have been clearly defined (Table 1). The region with the highest rate of isolation of H5N1 virus strains completely overlapped the Black Sea coast and wet territories connected with Via Pontica in the administrative districts of Dobrich, Varna and Bourgas. Only one H5N1 strain was isolated in 2006 in the Vidin region as a result of the migratory movement of swans infected with this viral type originating from the Danube river delta. The preserved territory of Poda covers a surface of 2 270 hectares. It is located on the south of Bourgas, close to and connected with the Black Sea. Two hundred and forty-five bird species occur in the region, some of them being included in the Red Book of Bulgaria and some of them of European importance. Many migrating and wintering species are found. The territory is on the migration route Via Pontica.

The first case of HPAI in wild birds was found in a dead mute swan found on the river Danube near the town of Vidin. It was confirmed as H5N1. This case was likely the result of spread by H5N1-infected migrating swans and wild ducks from the Danube delta. Mute swans were also found dead around Lake Durankulak at the coast in Kramorie, Burgas as a consequence of AI-H5.

The Bulgarian National Reference Laboratory on Avian Influenza and Newcastle disease in birds also holds several LPAI isolates:

- influenza A/H10N7/Mallard/ Montana/08., isolated from the fresh faeces of live mallards, with no clinical signs inhabiting the river Ogosta in the district of Montana;
- influenza A/H7N7/Mallard/ Han Krum/08, isolated from the internal organs of a shot mallard, inhabiting the river Kamchia, near Han Krum village in the district of Shumen.

Areas and Species at Risk

Based on AI cases in Bulgaria over the last three years, several regions can be identified: highly pathogenic viruses of the subtype H5N1 and low pathogenic subtypes H4, H6, H7 and H10.

The risk zones of AI penetration are connected with the wet areas and territories via the main migratory path of wild birds and the Black Sea coast (Figure 1). The national early warning and surveillance system was adapted to and covered these risk areas on the basis of periodic risk assessment.

Two well equipped laboratories in Sofia and Varna cover the needs for sample investigation and research activities connected with AI in the country. The national surveillance plan includes domestic and wild bird populations and domestic small and large scale poultry production in the country.

Based on risk assessment, we believe that future research should focus on the populations of several species of wild migratory ducks wintering at Shabla Lake (district of Dobrich), Varna-Beloslav Lake (district of Varna) and the wetlands of Poda connected to the Mandra Lake (district of Burgas). Surveillance will include marking of caught birds to monitor their AI status in case they are caught again. These three lakes have been selected based on the advice of the Bulgarian Society for the Protection of Birds (BSPB) Varna, and on the basis of their hydrologic features which make placing of ornithological nets for catching ducks possible. Other lakes do not allow access by boat or they freeze over in the winter.

Geographic coverage

**Shabla Lake Complex**

Area: 3 195 ha; altitude: 0–40 m.

Shabla lake (Dobruch district) one of the favorite places where wild birds stop when they migrate. The lake complex includes the lakes of Shabla, Ezerets and Shabla Tuzla, located over Sarmatian limestone in northeastern Bulgaria, 5 km northeast of the town of
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Shabla. Shabla Lake unites two closely located coastal firth lakes — Shabla and Ezerets — connected through an artificial canal. On the east, the lake is separated from the sea by a 30–50 m sand strip. Shabla Tuzla is a semi-saline lagoon, located 1.5 km southeast of Shabla Lake and separated from the sea by high dunes. The territory of Shabla Lake complex supports 259 bird species and is of strategic importance for the globally threatened red-breasted goose in winter, as, together with Durankulak Lake, it holds almost the entire global population of this species. The lake is one of the sites with considerable concentrations of whooper swan and mallard.

Varna- Beloslav Lakes Complex
Area: 4 681.8 ha; altitude: 0–101 m.

The complex includes two lakes, Varna and Beloslav, connected by an artificial canal and located to the west of the city of Varna. Varna Lake is a coastal firth lake of natural origin, although Beloslav Lake was a closed freshwater firth until 1923. Due to the digging of artificial canals connecting Varna Lake with the Black Sea and another one between the two lakes, the water salinity increased. Since lakes do not freeze in winter, they are preferred as a wintering site by different ducks, cormorans and other waterfowls.

Mandra-Poda Complex, District of Burgas
Area: 5 988 ha; altitude: 0–101 m.

The complex includes Mandra Lake with its adjacent wetlands. Mandra Lake is located at the Black Sea coast and is the southernmost of the Burgas lakes. Its northeastern part touches on the city of Burgas. This former semi-saline lake has been turned into a freshwater reservoir. A lagoon covering the areas of Poda and Uzungeren has been preserved between the reservoir wall and the Black Sea (Roberts, 1978). The complex has international importance for the regular wintering of up to 69 000 waterfowl belonging to 82 species.

Bourgas lake system (Mandra Dam, area Poda and Vaya Lake)
The areas with the highest number of isolated subtypes H5N1 virus of AI cover almost entirely a strip along the Black Sea coast and wet territories under Via Pontica in the areas of Dobrich, Varna and Bourgas.

Lake system and wet territories in Varna and Dobrich districts
Two findings were identified as important for AIV infections based on the epidemiological data. First, the availability of water reservoirs, some of which are warm waters being located near to electric power stations; and second, the availability of farms with ducks which are stocked with one-day chicks imported from France or produced from imported breeding eggs delivered by the same suppliers. Ducks are raised extensively in the first three months and afterwards are transferred to fattening farms with relatively good biosecurity standards.

Epidemiology and Surveillance
As shown in Table 1, isolated low pathogenic viruses from ducks were of subtypes H4 and H6 in the areas of Plovdiv, Pazardjik and Haskovo. Raising ducks for liver production on such farms is a popular practice in south and southeast Bulgaria (regions of Yambol, Sliven, Stara Zagora, Bourgas, Haskovo).

Table 1. AIVs isolated in Bulgaria during 2005–2008.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Date</th>
<th>District</th>
<th>Location</th>
<th>Sample</th>
<th>Bird species</th>
<th>Virus isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/malard duck/05</td>
<td>11.11.2005</td>
<td>Bourgas</td>
<td>Poda dam</td>
<td>Faecal sample</td>
<td>Malard duck</td>
<td>H6N2</td>
</tr>
<tr>
<td>A/swan/Vidin/06</td>
<td>31.01.2006</td>
<td>Vidin</td>
<td>Danube river</td>
<td>Internal organs</td>
<td>Swan</td>
<td>H5N1</td>
</tr>
<tr>
<td>A/swan/Varna/06</td>
<td>06.02.2006</td>
<td>Varna</td>
<td>Tzonevo dam</td>
<td>Internal organs</td>
<td>Swan</td>
<td>H5N1</td>
</tr>
<tr>
<td>A/swan/Kraimorie/06</td>
<td>07.02.2006</td>
<td>Bourgas</td>
<td>Kraimorie</td>
<td>Internal organs</td>
<td>Swan</td>
<td>H5N1</td>
</tr>
<tr>
<td>A/swan/Dobrich/06</td>
<td>06.2.2006</td>
<td>Dobritch</td>
<td>Durankulak Lake</td>
<td>Internal organs</td>
<td>Swan</td>
<td>H5N1</td>
</tr>
<tr>
<td>A/swan/Bourgas/06</td>
<td>17.02.2006</td>
<td>Bourgas</td>
<td>Chengenez Skale village</td>
<td>Internal organs</td>
<td>Swan</td>
<td>H5N1</td>
</tr>
<tr>
<td>A/mule duck/Parvomay/06</td>
<td>25.04.2006</td>
<td>Plovdiv</td>
<td>Parvomay town</td>
<td>Cloacal swab</td>
<td>Mule duck</td>
<td>H6N5</td>
</tr>
<tr>
<td>A/mallard/Pazardjik/06</td>
<td>21.03.2006</td>
<td>Pazardjik</td>
<td>Kovatchevo village</td>
<td>Faecal samples</td>
<td>Malard duck</td>
<td>H4N6</td>
</tr>
<tr>
<td>A/mule duck/Rajevo konare/07</td>
<td>14.05.2007</td>
<td>Plovdiv</td>
<td>Rajevo konare village</td>
<td>Cloacal sample</td>
<td>Mule duck</td>
<td>H4N6</td>
</tr>
<tr>
<td>A/malard/Krepost/07</td>
<td>18.04.2007</td>
<td>Haskovo</td>
<td>Krepost village</td>
<td>Cloacal sample</td>
<td>Mule duck</td>
<td>H4N2</td>
</tr>
<tr>
<td>A/mule duck/Rajevo konare/07</td>
<td>22.11.2007</td>
<td>Plovdiv</td>
<td>Rajevo konare</td>
<td>Cloacal swab</td>
<td>Mule duck</td>
<td>H6N5</td>
</tr>
<tr>
<td>A/mallard/Chan Krum/08</td>
<td>31.01.2008</td>
<td>Shoumen</td>
<td>Chan Krum village</td>
<td>Internal organs</td>
<td>Malard duck</td>
<td>H7N7</td>
</tr>
<tr>
<td>A/mallard/Montana/07</td>
<td>31.01.2008</td>
<td>Montana</td>
<td>Ogosta river</td>
<td>Faecal sample</td>
<td>Malard duck</td>
<td>H10N7</td>
</tr>
</tbody>
</table>

Figure 1. Areas in which there is a high risk of HPAI in Bulgaria.
From an epidemiological standpoint, control over the circulation of AIVs among duck flocks, especially before their gathering in bigger farms for fattening, is mandatory but often overlooked by official authorities due to lack of sufficient resources. During the first three months of rearing the ducks are exposed to high risks of contact with other domestic and wild birds, including migrating waterfowl. If researched, the study will ensure monitoring of a very important factor for circulation and ecological migration of AIVs in the country. Understandably, it will help the local duck industry (a major export earner for the country) to improve its biosecurity standards.

In the past few years the surveillance programme of the Bulgarian National Veterinary Service has included both passive and active surveillance of wild birds. In fact, mainly dead birds were submitted to laboratories, and very rarely faecal samples from places the wild birds inhabit. Also, attempts had not been made either to catch live wild waterfowl, or to monitor the status of marked birds and assess their potential for carrying of AIVs during different life stages. For that reason active surveillance and using microsatellite markers for bird migration tracing will improve the early warning and preventative AI control system.

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The currently ongoing outbreaks caused by HPAIV of the subtype H5N1 are of concern not only to the poultry industry but also to public health. This virus, which causes a high fatality rate among infected patients, may adapt to efficient human-to-human transmission and thus initiate a new human influenza pandemic (Alexander, 2000; BirdLife International, 2004; Wallenstein et al., 2007). Since 1996, when the ancestor virus was identified in domestic geese from China, outbreaks have spread and now encompass countries in Asia, the Middle East, Europe, and Africa. This spread of HPAIV among poultry flocks is traditionally thought to occur by transport of infected poultry, contaminated equipment, and persons associated with the poultry industry. HPAIV has occasionally been detected in wild birds near affected poultry flocks, but these birds have had limited or no role in virus dissemination. In the current outbreaks, however, wild birds are suspected of playing a major role as long distance virus vectors.

During the expansion of HPAI (H5N1) outbreaks from Asia to Europe, two events implicated wild birds, particularly water birds, as long distance virus vectors. First, virus outbreaks in 2005 spread rapidly westward from Russia and Kazakhstan in July and August to Turkey, Romania, and Ukraine in October. Wild water birds were suggested as vectors because the virus spread through areas that had no previous record of any virus presence and coincided with the fall migration of wild water birds between these areas. Second, at the beginning of 2006, HPAIV (H5N1) was detected in many wild water birds in western Europe, often in areas where no outbreaks had been detected among intensively surveyed poultry; this event overlapped with unusual water bird movements associated with cold weather in the Black Sea.

To prevent further spreading of H5N1, surveillance in domestic poultry as well as in wild birds should be strengthened in countries at immediate risk, especially along migrating bird routes. Resources should be focused on the reduction of close contacts between humans, domestic poultry and wildlife through better management practices and improved biosecurity in poultry production enterprises, especially those that are small and ‘open-air’ - where domestic poultry and waterfowl are allowed to mingle with wild birds.

Official competent authorities such as the Chief Veterinary Officer would also need to monitor ‘wet’ and wildlife markets, where wild and domesticated species are kept in close proximity and are at risk of exposure to a wide range of pathogens. Limiting contact with wild birds should therefore be part of any AI control strategy. The control

<table>
<thead>
<tr>
<th>Species</th>
<th>Size of wintering population on Shabla Lake</th>
<th>Size of wintering population on Varna-Beloslav Lake</th>
<th>Size of wintering population on Poda Lake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ruddy Shelduck</td>
<td>1–27</td>
<td>0–8</td>
<td>1–6</td>
</tr>
<tr>
<td>Common Shelduck</td>
<td>1–20</td>
<td>1–43</td>
<td>8–641</td>
</tr>
<tr>
<td>Eurasian Wigeon</td>
<td>3–550</td>
<td>3–112</td>
<td>20–3 530</td>
</tr>
<tr>
<td>Gadwall</td>
<td>8–15</td>
<td>2–15</td>
<td>19–102</td>
</tr>
<tr>
<td>Common Teal</td>
<td>4–408</td>
<td>15–336</td>
<td>175–3 700</td>
</tr>
<tr>
<td>Mallard</td>
<td>80–62 210</td>
<td>97–4 004</td>
<td>268–11 883</td>
</tr>
<tr>
<td>Northern Pintail</td>
<td>3–19</td>
<td>1–8</td>
<td>3–57</td>
</tr>
<tr>
<td>Garganey</td>
<td>3–14</td>
<td>18–81 at the time of migration</td>
<td>Up to 1 112 at the time of migration</td>
</tr>
<tr>
<td>Northern Shoveler</td>
<td>6–42</td>
<td>2–35</td>
<td>30–1 109</td>
</tr>
<tr>
<td>Red-crested Pochard</td>
<td>4–32</td>
<td>1–28</td>
<td>1–54</td>
</tr>
<tr>
<td>Common Pochard</td>
<td>36–3 520</td>
<td>1 125 – 10 240</td>
<td>371–1 3170</td>
</tr>
<tr>
<td>Ferruginous Duck</td>
<td>Up to 88 at the time of migration</td>
<td>0–2</td>
<td>4–45</td>
</tr>
<tr>
<td>Tufted Duck</td>
<td>16–735</td>
<td>75–2408</td>
<td>486 – 12 800</td>
</tr>
<tr>
<td>Greater Scaup</td>
<td>2–26</td>
<td>-</td>
<td>5–100</td>
</tr>
<tr>
<td>Longtailed Duck</td>
<td>3–6</td>
<td>-</td>
<td>0–3</td>
</tr>
<tr>
<td>White-winged Scoter</td>
<td>-</td>
<td>-</td>
<td>2–12</td>
</tr>
<tr>
<td>White-headed Duck</td>
<td>-</td>
<td>3–5</td>
<td>24–202</td>
</tr>
<tr>
<td>Black Scoter</td>
<td>-</td>
<td>-</td>
<td>0–3</td>
</tr>
</tbody>
</table>
PRESENT AND FUTURE NEEDS

Monitoring, sampling and analysis of the viral subtypes of AI found in wild birds needs to be done in order to fully understand their role in the propagation and spread of HPAI viruses. Multidisciplinary research is required that brings in the competencies of veterinarians, wildlife specialists, ornithologists, virologists, molecular biologists and other specialities. Besides the current regional and country specific AI projects being implemented by FAO, Mongolia has been assisted through a regional technical co-operation to review emergency preparedness and surveillance activities for HPAI since the outbreak in wild birds was reported.

A Global Strategy for the prevention and control of HPAI has been prepared by FAO and OIE under the umbrella of the Global Framework for the Control of Transboundary Diseases (GF - TADs). This Global Strategy addresses country level activities as well as the indispensable regional and international coordination. Within the epidemiological context of the current HPAI outbreaks, there is an urgent need to strengthen the joint FAO/OIE/WHO Global Early Warning System (GLEWS) so as to improve the regional capacity for early detection and response to AI incursions. Immediate support to national Animal Health Services will be required in Eastern Europe for emergency preparedness, surveillance and early response activities. Diagnostic capability in the region for avian influenza have been substantially enhanced by national efforts, with coordination and support provided by international organization. However, surveillance methods are still seriously inadequate to allow confident national and regional decisions to be made.

Specifically, surveillance efforts should be shifted from simple case-finding to identification of risk factors influencing maintenance of infection, and integrate surveillance procedures into control strategies. Also, for each country, the transmission pathways which are considered to have been responsible for infection dissemination in the current epidemic should be identified, and a surveillance strategy developed which will allow each of these pathways to be monitored, and changes in prevalence to be assessed. For example, market surveillance and interviews should be used to identify high-risk markets, bird types and bird sources, and control strategies built around this information. Further, for each country which no longer has active transmission, monitoring and sampling efforts should be directed to detect a new incursion should be determined within the limits of available resources.

Other steps would include applying nuclear molecular epidemiological investigation methods more comprehensively, to clarify epidemiological processes which are influencing the evolution of the epidemic: assessing the level of human exposure under various circumstances, in order to evaluate the risks of emergence of a virus capable of human to human transmission, and use this information to help guide the allocation of resources to different elements of the control strategy. Further needs include developing rapid and standardised methods for the routine analysis of surveillance data which would identify important changes in the H5N1 situation, and enable notification of this information to the competent authorities; and harmonised collection and presentation of surveillance and control data across countries in the region, so that information can be interpreted in a compatible way across the region.

CONCLUSIONS

The region with the highest rate of isolation of H5N1 virus strains completely overlapped the Black Sea coast and wet territories connected with the Via Pontica migration pathway in the administrative districts of Dobrich, Varna and Bourgas. Isolated LPAI strains from ducks were of subtypes H3, H4 and H6 in the areas of Plovdiv, Pazardjik, St. Zagora, Yambol, Sliven and Haskovo.

Raising ducks for liver production is a popular practice in south and southeast Bulgaria. From an epidemiological standpoint, control over circulation of AVs among duck flocks, especially before their gathering in larger farms for fattening is a mandatory requirement of official authorities. To prevent the spread of HPAI, surveillance of domestic poultry as well as wild birds should be strengthened in countries at risk, especially along bird migration routes. Monitoring, sampling and analysis of the viral subtypes of avian influenza found in wild birds needs to be done in order to fully understand their role in the propagation and spread of HPAI viruses.

REFERENCES


Vitamin D Metabolism in Experimental Animals: Kinetics of Solanum glaucophyllum Active Principle in Cows and Assessment of Calcium, Phosphorus and Vitamin D3 Requirements in Broilers

M. Dallorso1*, J. Azcona2, B. Iglesias2, S. Gil3 & N. Belmonte1

ABSTRACT

In 1990 our group began working on the development of a sensitive method to measure the active principle (1,25 dihydroxy-vitamin D3-glycoside) of Solanum glaucophyllum, a plant which grows wild in Argentina and causes calcinosis in breeding cattle. A radioreceptor assay (RRA) was applied to measure the free vitamin D metabolite in the plasma of experimental cows that were fed the plant in order to study the kinetics of the active principle. The 1,25 dihydroxyvitamin D concentration in plasma showed a 33-fold increase four h post treatment. Peak levels were recorded 12 h after dosing, decreased by half between 24–36 h and continued declining until 48 h. More recently, this plant has been proposed as a source of vitamin D activity (VDA) and thereby may contribute to improving Ca and P utilisation by animals and environmental care. The effects of different dietary levels of calcium (Ca) and phosphorus (P) over the range between commercial recommendations (control) and two thirds of NRC requirements (basal) as well as different sources of those minerals were therefore studied in experiments covering either a part or the entire breeding cycle of broilers through measurements of productive, nutritional, skeletal and biochemical parameters. Results indicated that birds fed diets deficient in these minerals exhibited skeletal responses but nevertheless showed better productive responses than those fed control diets. The high levels of vitamin D3 employed in commercial farms (25 times NRC recommendations) could enable birds fed on deficient diets to increase synthesis of the active metabolite of the vitamin in order to partially overcome deficiencies in these minerals. On the other hand, such high levels of vitamin D3 might have been unbalanced for optimal efficiency, at least under the experimental farm conditions of the present work.

INTRODUCTION

Enteque Seco (ES), which affects grazing cattle in Argentina and other countries in South America, is caused by ingesting fallen leaves of the toxic weed Solanum glaucophyllum (SG) (Okada et al., 1977). It is characterised by loss of body weight, kyphosis, stiffness of the forelimbs, soft tissue calcification and modifications in Ca and P plasma concentrations (Worker and Carrillo, 1967). The active principle in SG is 1,25(OH)2vitamin D3-glycoside (Haussler et al., 1976). The economic relevance of SG could be seen on the one hand as being the causal factor of toxicosis of grazing cattle (negative value), and on the other, as a valuable source of vitamin D3 active metabolites (positive value). Until now, most scientific work has dealt with the pathology and biochemistry of this calcinotic disease in experimental animals. Conversely, only a few publications have described quantitative studies about the vitamin D synthetic capability of the plant (Weissenberg, 1989), and only one report could be found about the environmental conditions that might influence vitamin D3 production by the plant (Puche et al., 1980) in addition of the recent report of Dallorso et al. (2008).

Pharmacological applications of SG have been reported in human and veterinary medicine as well as in animal production. Potential veterinary uses include the prevention of milk fever, pseudo-vitamin D deficiency of pigs and acidosis in chicks (Weissenberg, 1989). The first assay applied in animal husbandry was about elucidating the effect of SG powder from leaves on egg shell quality in laying hens (Gallego et al., 1978/9). Recently, SG has been used as an additive in the feed of chicks to improve phosphorus utilisation (Cheng et al., 2004), and a further promising application would be the supplementation of finishing cattle with SG to improve meat tenderness (Paaren, 1999).

The present report summarises some unpublished results of research to improve the diagnosis of ES using a radioreceptor assay (RRA) to determine the concentration of 1,25(OH)2vitamin D in plasma (1,25D) based on the knowledge that 1,25vitamin D3-glycoside is cleaved by intestinal bacterial enzymes to 1,25D (Boland et al., 1987). This enabled us to apply our experience and expertise to the area of Ca and P nutrition in poultry.
MATERIALS AND METHODS

Studies on ES Diagnosis
The experiment was performed to describe the fate of the active principle in SG on experimental cows.

Animals
Two non-pregnant, non-lactating adult Jersey cows without any clinical and pathological signs were used. Animals were housed indoors in individual pens and fed diets supplying 75 g Ca and 35 g P/d. Cows (‘A’ and ‘B’) were fed a diet composed of 65% corn silage, the remainder being a mixture of beet pulp, soybean meal, distillers grain and alfalfa hay with a commercial supplement. Each animal was fed 9 kg dry matter/d.

Vitamin D Compounds
The [3H]-1,25D3 was generously supplied by the National Animal Disease Center, (NADC), Iowa, USA. The 1,25D3, 1,24,25(OH)3D3; 1,25,26,(OH)3D3 and 1a, 25(OH)2D3–26,23 lactone were kindly provided kindly by Dr. Milan Uskokovic (Chemical Research Department, Hoffmann-La Roche, Inc., N.J., USA).

Solanum glaucophyllum (SG)
The SG was provided by Dr. C. Corbellini (INTA, Argentina). The leaves were collected in Mercedes, Bs. As., Argentina, in May 1995.

Experiment
After 2 weeks on the experimental diet a single dose of 100 g of powdered dried leaves of SG was mixed with the silage and given to each of the two cows. Two blood samples were taken 24 h before feeding and just before the single dose of SG to obtain baseline physiological data. Sampling was then continued every four h during the first d, at 36 h, 48 h, 72 h, 96 h, and on d seven after dosing. Blood samples were taken from the jugular vein with heparinised syringes, centrifuged to obtain plasma, and kept frozen at –90 ºC until analysed.

Determination of 1,25D3
A one-step extraction procedure was used (Dallorso et al., 2001) followed by analysis using a non-HPLC charcoal binding assay using thymus gland receptor (Reinhardt et al., 1984). Radioactivity was followed by analysis using a non-HPLC charcoal binding assay using thymus gland receptor (Reinhardt et al., 1984). Radioactivity was quantified using a scintillation counter (Beckman L.S. 8 000).

Determination of 1,24,25(OH)3D3, 1,25,26,(OH)3D3 and 1,25(OH)2D3–26,23 Lactone
To elute the three more polar vitamin D3 metabolites, prewashed SPE C18 cartridges were sequentially washed after sample application with double distilled water, methanol:water (50:50), hexane:methylene chloride (90:10), and hexane:isopropanol (99:1). Finally, these three metabolites were eluted with hexane:isopropanol (85:15). To obtain the three metabolites separately, the eluates were evaporated in a vacuum centrifuge, hexane:methylene chloride (2/1): isopropanol:methanol (46:50:4) added, injected onto a HPLC unit (Waters Corp.) and run through a silica column, with monitoring using an absorbance detector (Model 440) at 254 nm. The mobile phase used was hexane:methylene chloride (2/1): isopropanol:methanol (46:50:4). Each metabolite was collected in a different fraction by an ISCO Collector (Model 568) and all were analysed by radioimmunoassay (RIA, Hollis et al., 1996). The antibodies were raised in rabbits in the NADC. The [3H]-1,25D3 fraction was used to determine the recovery of all the vitamin D metabolites.

Experimental Studies on Calcium and Phosphorus Nutrition in Poultry
Intensive poultry production produces an excess of P in manure. When this is applied on fields as fertiliser, the surplus can cause eutrophication of water bodies (Sharpley et al., 1994). In order to contribute to environmental care through nutritional management the effects of different dietary levels and sources of Ca and P covering the range between commercial recommendations and near two-thirds of NRC (1994) requirements on productive, nutritional, skeletal and biochemical responses were studied in a series of experiments covering part or the entire breeding cycle of broilers. Two of these experiments are presented.

Animals
These were males Cobb–500 from Granja Tres Arroyos, Capilla del Señor, Buenos Aires, Argentina.

Trial 1
This covered the period from 7–20 d of age (initial weight [d 7] was 151±17 g). The feed was mash composed of corn, soy meal (40% crude protein), soybean, soy oil, limestone, calcium phosphate, salt, methionine and a premix containing 5 000 IU vitamin D3/ kg of diet and covering requirements for this strain of broilers except those of Ca and P in the basal diets. Animals were divided into two groups each of 25 broilers i.e. a control group that received Ca = 0.92% and P= 0.41%; and a group receiving a basal diet with 0.56% Ca and 0.28% P.

Trial 2
This was carried out when animals were between 1 and 49 d of age. The feed was mash composed of corn, soy meal (45% crude protein, soybean, meat meal (41% crude protein), soy oil, limestone, salt, methionine, lysine, threonine and premix containing 5 000 IU of vitamin D3/ kg diet and covering requirements for this strain of broilers except for Ca and P levels in basal diets. The control animals (five pens of 15 animals each) were on a starter ration of 0.9% Ca and 0.45% P, a grower diet with 0.88% Ca and 0.42% P, and a finisher diet with 0.84% Ca and 0.40% P during the last week. The diet had 0.78% Ca and 0.35% P. Animals on the basal diet (five pens of 15 animals each) were on a starter ration containing 0.56% Ca and 0.28% P; a grower diet with 0.55% Ca and 0.26% P and finished on a diet with 0.52% Ca and 0.25% P. During the last week, the diet contained 0.49% Ca and 0.22% P.

Measurements
Animals were weighed and their feed consumption measured weekly and data were expressed as the average of each pen. At the end of each trial broilers from each pen (n = 5) were taken at random and maintained in cages until euthanised by complete bleeding to be used for some of the following determinations:
- Ca, P and 1,25D levels in plasma obtained from heparinised blood samples that were immediately centrifuged at 3 500 rpm for 10 min;
- Ca, P and ash levels, morphology, weight, density and volume of right tibiotarsi; and
- Ca, P and ash in feed and manure.
Feed samples of each treatment were taken from the appropriate bags after preparation. Manure samples were collected in cages during a period of 8 h prior to euthanasia. Ca was determined by atomic absorption spectroscopy (AAS) on plasma diluted in a solution containing LaO2 0.5% and P2O5 by colorimetry (Fiske and Subbarow, 1925). Bone volume was determined by complete immersion of tibiotarsi in a fixed volume of water in a graduated tube (bone volume (mL) = water volume with bone inside the tube - water volume without bone inside the tube). Bone density was calculated as bone weight in air/bone volume. Tibiotarsi, feed and manure were dried in an electric stove at 80ºC (DM), incinerated at 580ºC and ash dissolved in HCl: H2O (1:1) and filtered, and washed with water to a final volume of 50 mL.

[1,25D] was analysed by RIA (Gil and Dallorso, 2002) using plasma samples prepared as described previously (Dallorso et al., 2001). A radioreceptor assay (RRA) was replaced advantageously by RIA to determine the active vitamin D metabolite due to the fact that the binding capacity of the antibody raised against 1,25D3 remains constant when stored in solution at –20 ºC while the intracellular vitamin D receptor (VDR) employed in RRA, although freeze-dried and stored at –80 ºC needs to be prepared frequently, among other disadvantages. Although the antibody binds to 25(OH) vitamin D (25D) with five percent cross-reactivity (100% 1,25D), since 25D was undetectable in the analyte fraction (F2) and conversely, the analyte with our previous findings (Dallorso et al., 1994). It was also observed that in addition to 1,24,25D3, the metabolite 1α-lactone was also a major circulating 1,25D3 form. This is in agreement with reports of pharmacological doses of 1,25D3 to adult cows (Horst et al., 1983) and with experiments with radio-inert compounds in rats that showed injected 1,25D3 to be an efficient precursor of 1α-lactone (Horst et al., 1984). No significant elevation was recorded in plasma 1,25,26D3 levels. This situation is in accordance with previous work (Reinhardt et al., 1982). It seems reasonable to conclude that 1,24,25D3 and 1α-lactone arose from further metabolism of the increased 1,25D3 levels emanating from the treatment with SG since these compounds circulate in low concentrations in normal cows (Reinhardt et al., 1981) and are found to be elevated in different species like the rat (Ohnuma and Norman, 1982), dog (Ishizuka et al., 1984) and cow.

Analysis of data
In trial 1, animals were considered experimental units (n = 25 animals in each group) for the analysis of individual variables under study. Values of body weight were studied in all 25 animals. The other determinations were made on a randomised sample of five animals. In trial 2, pens were considered experimental units (n = 5 pens in each group of 15 animals each). Values of body weight and feed consumption were obtained for all 15 animals in each pen and expressed as an average of each pen. The other determinations were made using a randomised sample of six animals from each pen.

Differences between control and basal groups were detected by Two-Sample t test (P-value ≤ 0.05) using the software Statistix SXW-Version 8.0.

RESULTS AND DISCUSSION

Studies on ES Diagnosis
The evolution of the levels of vitamin D metabolites in cows ‘A’ and ‘B’ is shown in Figures 1 and 2. The 1,25D concentration showed a 45-fold increase to 3 345 pg/mL by 4 h post treatment in cow ‘B’ and a 20-fold increase to 5 200 pg/mL in cow ‘A’. The maximum was observed 12 h after feeding in both cows, decreased to half its maximum between 24 h and 36 h and continued declining until 48 h. The 1α-lactone, 1,24,25(OH)3D3 and 1,25,26(OH)3D3 concentrations were quantified before and at 24, 48, 72, and 96 h and on day 7 post feeding. The 1,24,25(OH)3D3 and 1α-lactone levels reached a maximum at 24 h with 9.7 and 14-fold increases (1 930 pg/mL and 1 037 pg/mL) respectively in cow ‘B’ (see Figure 2). The 1,25,26(OH)3D3 concentration was only slightly elevated compared with the other three vitamin D metabolites. All three metabolites returned to near baseline levels by 7 d. As expected, there was an increase in 1,25D and 1,24,25D3 levels after feeding cows with SG which is consistent with our previous findings (Dallorso et al., 1994). It was also observed that in addition to 1,24,25D3, the metabolite 1α-lactone was also a major circulating 1,25D3 form. This is in agreement with reports of pharmacological doses of 1,25D3 to adult cows (Horst et al., 1983) and with experiments with radio-inert compounds in rats that showed injected 1,25D3 to be an efficient precursor of 1α-lactone (Horst et al., 1984). No significant elevation was recorded in plasma 1,25,26D3 levels. This situation is in accordance with previous work (Reinhardt et al., 1982). It seems reasonable to conclude that 1,24,25D3 and 1α-lactone arose from further metabolism of the increased 1,25D3 levels emanating from the treatment with SG since these compounds circulate in low concentrations in normal cows (Reinhardt et al., 1981) and are found to be elevated in different species like the rat (Ohnuma and Norman, 1982), dog (Ishizuka et al., 1984) and cow.

Figure 1. Plasma 1,25(OH)2D3, 1 alpha lactone; 1,24,25(OH)3D3 and 1,25,26(OH)3D3 concentrations after a single oral dose of 100 g Solanum glaucophyllum to cow ‘A’.

Figure 2. Plasma 1,25(OH)2D3, 1 alpha lactone; 1,24,25(OH)3D3 and 1,25,26(OH)3D3 concentrations after a single oral dose of 100 g Solanum glaucophyllum to cow ‘B’.

Analysis of data
In trial 1, animals were considered experimental units (n = 25 animals in each group) for the analysis of individual variables under study. Values of body weight were studied in all 25 animals. The other determinations were made on a randomised sample of five animals. In trial 2, pens were considered experimental units (n = 5 pens in each group of 15 animals each). Values of body weight and feed consumption were obtained for all 15 animals in each pen and expressed as an average of each pen. The other determinations were made using a randomised sample of six animals from each pen.
Horst et al., 1983) treated with vitamin D derivatives. The appearance of each metabolite paralleled the appearance of 1,25D.

The significantly augmented 1α-lactone or 1,24,25D3 values caused by the 1,25D3 degradation recorded after the feeding period cannot be used to diagnose SG toxicity. In previous work with rabbits and ewes dosed orally with SG (Dallorso et al., 2000a), high plasma levels of the 1,25D metabolite occurred 1 h after administration and remained higher than control animals for up to 24 h after administration. Also, while rabbits dosed orally and subcutaneously showed increments in the plasma metabolite, highest values were recorded in those dosed orally. The calcinotic effects seen in rabbits dosed subcutaneously could have been caused by the 1,25D3-glycoside without the intervention of enteric microbial enzymes (Dallorso et al., 2000b). This was suspected earlier (Barros et al 1981) who showed specific effects of this active principle in the aorta of rabbits 6 h after intravenous administration of aqueous extracts of SG leaves.

**Experimental Studies on Calcium and Phosphorus Nutrition in Poultry**

There were no significant differences (P = 0.6819) in body weight at 20 d of age between control (746.5 ± 62.47 g) and basal (736.0 ± 100.86 g).

![Calcemia](image)

![Percent Dry Matter of Right Tibiotarsi](image)

![Percent Ash of Right Tibiotarsi](image)

![Calcium Concentration in Right Tibiotarsi](image)

![Phosphorus Concentration in Right Tibiotarsi](image)

![1,25(OH)2vitamin D plasmatic concentrations in broilers](image)

**Figure 3.** Biochemical and skeletal variables measured at 20 d of age in broilers (Trial 1).
groups in Trial 1 although the controls had higher body weights. Additionally, feed conversion values (g feed consumed / g weight gain) were lower in the controls (1.998 vs 2.099). At 20 d of age lower values (P ≤ 0.05) were recorded in the basal groups for plasma Ca levels, percentage dry matter and ash, and for Ca and P levels of the right tibiotarsi (Figures 3a-f). Levels of vitamin D active metabolite, were twice as high in the basal than in the control group, but the difference was not significant (P = 0.273) (see Figure 3e). Neither were significant differences detected between the groups in plasma Pi concentrations, fresh and dry weight, and the volume and density of right tibiotarsi. Ca and P levels in manure paralleled those in feed (data not shown).

In Trial 2, although animals of the basal group were fed only two thirds of the recommended levels of Ca and P during their entire breeding cycle they showed significant and favourable differences in body weight at 21 d and 28 d of age, and in feed conversion at 21, 28 and 35 d of life (Table 1). Also, significantly lower values (P ≤ 0.05) for ash and Ca levels accompanied by higher widths of the distal epiphysis and the right tibiotarsi were recorded at 49 d of age in these animals (Figures 4a–c). However, vitamin D active metabolite concentrations in plasma did not show significant differences (P = 0.594; n = 5) between the basal and control groups, but since the mean basal group value was slightly higher a greater number of experimental units should be used in future studies to ensure rigorous statistical analysis (Figure 4d). Other nutritional, biochemical and skeletal variables were similar in both groups.

The high levels of vitamin D3 employed here (25 times the 1994 NRC recommendations) and in commercial farms (Barroeta et al., 2002) could enable birds fed on basal diets to increase synthesis of the active metabolite of vitamin D in order to ameliorate partially the effects of Ca and P deficiencies. Conversely, the diet with high levels of vitamin D3 together with the recommended levels of Ca and P for lower levels of vitamin D3 (NRC, 1994) might have been
unbalanced for optimal efficiency, at least in the experimental farm conditions of the present work.

CONCLUSIONS
The determination of plasma 1,25D levels in experimental cows contributed to knowledge about calcinosis of cattle in Argentina, named ES. The results obtained demonstrated the rapidity with which the active principle of SG is hydrolysed by digestive enzymes in ruminants, evidenced by the augmented levels of the free vitamin D₃ metabolite circulating in blood four h after oral administration. The preliminary results obtained with feeding broilers suggest that it will be necessary to investigate the performance of commercial broiler chickens during the entire cycle with different combinations of vitamin D₃, calcium and phosphorus in order to determine the appropriate levels for optimum and deficient diets.

ACKNOWLEDGEMENTS
We are grateful to Dr. R. Horst and his team for their generous assistance in putting into practice the determination of vitamin D metabolites in our laboratory, to Dr. N. Carou (CNEA) for teaching us the basis of the RIA method; to Med. Vet. F. Lema (UNLZ) and Lic. E. Pawlak (CNEA) for their professional assistance; and to the students (UNLZ): M. S. Rovegno and M. V. Neme (2003–2006), for their technical assistance. The authors also acknowledge the support provided through the Programa de Becas Externas ‘R. H. Thallman’, NADC, USDA, ARS, Ames, Iowa, USA.

REFERENCES
Animal Health in Albania

A. Lika¹*

ABSTRACT

The animal health service policy in Albania represents an integral component of overall governmental, social and economic policy in the field of agricultural and rural development, public health, food processing and import/export of animal products. In order to obtain the necessary political, economic and public support, the animal health service attempts to contribute effectively to the overall development of the country which aims at improving the standards of living of its inhabitants. Practical means of contributing to national development include reducing food losses due to animal morbidity and mortality, increasing the productivity of the livestock population, protecting human health against zoonotic diseases and ensuring humane treatment of animals. An animal health strategy contributes to the creation of conditions necessary for uninterrupted animal disease surveillance and control in the country. The main animal health problem in Albania is brucellosis in ruminants, caused by B. melitensis. This infection currently affects the entire country, reaching a prevalence of 10% in several districts. The latest and most severe outbreaks of classical swine fever were identified on 1996 when 5 515 animals were infected and 3 683 animals died. The circulation of bluetongue virus (BTV) was detected for the first time in Albania in 2002 with a seroprevalence of 15%. The evidence of BTV circulation in Albania and the absence of the main vector C. imicola suggest that other Culicoides species could be implicated in virus transmission. H5N1 avian influenza in Albania was confirmed in March 2006 in backyard flocks in the villages of Cuke and Peze-Helmes. In both villages there were no human cases. Rabies was of concern in Albania from 1928 until 1976. The disease re-emerged in March 2001 in the village of Morine in Kukes district affecting a domestic dog and three persons were bitten. Other cases have been reported in northern Albania.

INTRODUCTION

Agriculture plays an important role in Albania, although currently it is practised largely at a subsistence level. Albania’s farming sector has been dominated by small private holdings since the collapse of the communist State in 1991, when peasant farmers disbanded the quasi-state collective farms. Agriculture subsequently became an important source of income support in rural areas, and is now undergoing a transition from a largely subsistence sector to a commercial one. Currently, the sector contributes 25% of GDP, which is high compared with neighbouring countries, while average gross income per farm is estimated at about US$ 1,800.

Around 40% of Albania’s 28,748 km² land area is classified as agricultural land (24% arable and 15% pasture). The rest is divided between forest (36%) and other uses. Over 75% of Albania is hilly and mountainous, and much agricultural land is hilly. Albania is predominantly mountainous in the north and east, with agricultural land concentrated in the more densely populated coastal plains of the west (43% of arable land). A further 34% of agricultural land lies in river valleys; 23% is upland. Albania is located in the Mediterranean climatic zone and has short winters and hot, dry summers. It has abundant precipitation (1,430 mm annual rainfall) concentrated in autumn and winter, with frequent droughts in summer. It also has extensive underground water resources (World Bank, 2007).

The total number of farms in Albania is approximately 370,000, mostly dominated by small farms (average size 1.14 ha according to official statistics, or 0.8 according to HBS data [Albanian Agriculture, 2007]. This is a much smaller average size compared with an average of 5 ha for Central and Eastern European countries and 27 ha for Western Europe. This is an important handicap to improving agricultural productivity and encouraging sustainable development of the agricultural sector.

MATERIALS AND METHODS

Identification of Gaps

The vision of the National Animal Health Programme (NAHP) is to improve the health and welfare of animals for meeting the needs of stakeholders, enable safe production of food, improve health of the public, sustain the rural society, and support the rural economy. Effective national food control systems are also essential to protect the health and safety of consumers. Food-borne diseases caused by microbiological contamination remain a major public health problem in Albania. The country is registering an increase in brucellosis, particularly in humans, transmitted either through contact with animal tissue or through the ingestion of contaminated milk and milk products (Figure 1)

The concept and requirements for working towards this vision are:

• the current veterinary services in the country, including the veterinary diagnostic institution, are very weak. The few resources in place are fragmented and reflect the historic paradigm of the previous regime with incomplete transition towards a market economy. The veterinary services in the 12 districts/regions appear to have been connected administratively but their field operations are neither connected with the national interest nor geared to the above vision;

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there is a need to link food safety and zoonotic aspects to the public health sectors so that public interest and funding resources can be increased;

- the livestock sector is undeveloped and consumers do not have much influence or organisation. The veterinary service should therefore take this opportunity to present a comprehensive plan for national animal health with benefits for both consumers through safety food and the livestock sector itself through better production and trade;

- budgets need to be in place to meet all needs including coordination with many of the international organisations to secure funding for specific activities within the comprehensive NAHP;

- the foundation of a reliable NAHP is a scientifically based surveillance system in which contingency planning is incorporated for specific health events.

Data Collection

Our data originated from the Department of Animal Health in the Food Safety and Veterinary Institute and direct contact with District Veterinary Services throughout the country.

RESULTS AND DISCUSSION

The Main Animal Diseases

**Brucellosis**

The main problem is brucellosis in ruminants, caused by *B. melitensis*, which is widespread in several districts of the country. The infection reached its highest levels between 1960 and 1965, and subsequently decreased through the implementation of different control measures. In 1989, the country was proclaimed free of bovine brucellosis, with a low prevalence (0.002%) in small ruminants. During this period, compulsory herd testing and removal of reactors was successfully implemented and helped to eliminate positive animals. Moreover, application of the B–19 strain vaccine in cattle in combination with animal tracing yielded significant results. After the political and economic changes in the 1990s, the infection spread in animals throughout the country and reached its climax in 2000. The uncontrolled movement of animals, the failure to apply sanitary and quarantine measures as well as the low level of cultural and technical education of farmers, together with a limited budget for implementing an eradication strategy (total screening, total elimination of positive heads), led to this expansion across the country. This infection currently affects the entire territory of the country, reaching a prevalence of 10% in several districts such as Saranda and Gjirokaster. The number of persons affected by brucellosis is increasing, particularly in rural areas (Kakariqi, 2006). A new strategy for the control of brucellosis in Albania using *B. melitensis* rev 1 strain vaccine was introduced in 2003, and is starting to have positive results.

Table 1. Number of pigs vaccinated against classical swine fever.

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<th>Year</th>
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<tr>
<td>1996</td>
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<td>1997</td>
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<td>2007</td>
<td>17 679</td>
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Figure 1. Geographic distribution of brucellosis. Incidence (cases/10 000 persons) according to district.
Classical Swine Fever
This was first diagnosed in 1966 in southeast Albania and outbreaks of the disease were later identified elsewhere based on clinical signs and postmortem lesions (it originated from swine imported from China). The disease was eradicated subsequently following drastic measures which included massive culling, quarantine, etc. The infection reappeared in 1975 in a reserve of wild boars in the south of Albania (Karaburun).

The latest and most severe outbreaks of the disease were identified in 1996 (using direct immunofluorescence test in the Food Safety and Veterinary Institute) when 5,515 animals out of 35,235 swine were infected and 3,683 animals died. The rest of the swine population was subjected to compulsory slaughtering and despite the very restricted financial possibilities, the State reimbursed the cost of the operation. The disease was kept under control by applying surveillance, quarantine, stamping out and vaccination.

In general there are now only sporadic outbreaks of infection in the north of the country, although vaccination is still applied in old outbreak areas (Table 1).

Bluetongue Virus (BTV)
This was detected for the first time in Albania in 2002 (Di Ventura et al., 2004) with a seroprevalence of 15% (Figure 2) determined by competitive ELISA and virus neutralisation assays. During that year a survey for Culicoides, was also made. Twenty species were identified in the collections (Figure 3). The finding that serotype 9 of the virus was the only one involved suggested that BTV infection came from neighboring countries. However, evidence of BTV circulation in Albania and the absence of the main vector C. imicola suggest that other Culicoides species could be implicated in virus transmission. The high abundance of the Obsoletus complex, from which BTV was recently isolated in outbreaks where no specimens of C. imicola were captured, also suggest that probably the vector belong to this complex (Goffredo et al., 2003).

H5N1 Avian Influenza
The first case in Albania was confirmed on 7th March 2006 in a dead chicken from a backyard flock in the village of Cuke in the Sarande district of southern Albania, and a further case was confirmed on 21 March 2006 in the village of Peze-Helmes near Tirana. Dead chicks were sent to the Animal Health Laboratory in Tirana where avian influenza virus was isolated on SPAF eggs and detected using haemagglutination inhibition (HI) and agar immunodiffusion (AGID) tests. The isolate was sent to the OIE Reference Laboratory in Weybridge, UK which confirmed the presence of H5 with a final typing of H5N1.

In both villages outbreaks were confined to backyard poultry and there were no cases in humans. Also, infections occurred on holdings with direct access to an adjacent floodplain and appear to have resulted from contact with infected migrating waterfowl. The level of contact may have been no more than some hens foraging on a pasture where waterfowl had grazed some time previously. The severity of the outbreak was limited by the fact that the majority of flocks in the village were either housed or confined within a walled garden. The response measures against AI during outbreaks in Cuke and Peze were well planned.

Following confirmation of the outbreak, the authorities put into operation the contingency plan which had been drawn up for such an eventuality. One of the first tasks was to conduct an awareness campaign among the villagers outlining the measures which would be implemented and the precautions that they should take. At this stage a preliminary appraisal was made of the number of poultry to be slaughtered and the manpower and resources required. A landfill site for the disposal of culled birds and contaminated material was identified and prepared. A number of meetings were held with per-
sonnel engaged in the operation where areas of responsibility were identified and delegated. Training was provided to hired workers on technical and safety aspects of culling and disinfection to ensure a professional and effective performance of these tasks.

**Quarantine**

A quarantine zone was established around the village with disinfection points for people and vehicles at the main access points. This action appeared to be very successful with a high level of compliance by all inhabitants. Within a three km zone, biosecurity measures were implemented including a requirement that all poultry must be housed and all illness and mortality in birds be reported to the veterinary service of the communes.

**Depopulation and Disinfection**

Culling and disinfection operations were carried out by three teams each under the supervision of a veterinarian. The teams consisted of a pickup truck and driver, a record keeper, two workers responsible for culling operations and one for disinfection. The teams had police support if required but most flock owners were very cooperative. Birds were killed by dislocation of the neck before being put in black plastic sacks. Initially it was debated whether it was necessary to kill the birds or let them die by suffocation. In practice killing proved both more humane and more efficient as it precluded any escape of birds from a torn bag in transit or at the landfill.

**Disposal of Dead and Diseased Birds**

The landfill was located about 2 km from the village. The site was well located being easily accessible from the village but isolated from any habitation. It was under constant supervision during the culling operation and was closed with topsoil at the end of each day to prevent scavenging by foxes or dogs.

**Rabies**

This was a disease of concern from 1928 until 1976. Cases were reported sporadically and most of them in wild animals (wolves, foxes, jackals, etc) and in stray cats and dogs. From 1976 up to 2000, rabies cases were minimal and the country was classified as free from disease, even if in neighbouring counties the disease was increasing. This status of ‘freedom from disease’ for that period can be explained by the fact that at the time the veterinary service was well organised and the border was protected by a permanent barrier called Clone 1. The disease reoccurred in Albania in March 2001 in Morine village in the Kukes district affecting a domestic dog and three persons were bitten who fortunately received the appropriate treatment quickly. Since then, further cases have been reported in northern Albania. Specifically:

- in November 2002 a fox was diagnosed positive in Qereti village in Puka district;
- in March 2003 a further two foxes were found positive in Gjorica village in Bulqiza district;
- in May 2004 a rabid wolf wandered around the villages of Perollaj, Helshan, and Zahrishte in the Has district. As a consequence of this incident, 21 animals of different species were bitten and about seven of them showed clinical symptoms; (Figure 4)

![Figure 4. Villages with cases of rabies (in red) from 2001-2006.](image)

- since then, no cases of rabies have been reported;
- the rabies virus was identified by brain histo-pathological findings and immuno-fluorescence microscopy on fresh brain tissues.

Control measures comprise:

- vaccination of remaining dogs in the village;
- disinfection of likely contaminated areas;
- enforcement of quarantine measures;
- suspension of trade in live animals and by-products;
- strict surveillance of remaining dogs in the village;

Rabies monitoring in animals is performed by the Food Safety and Veterinary Institute. Laboratory confirmation of rabies virus is based on positive results obtained by the direct fluorescent antibody test (IFAT) using antirabies test serum from ‘SIFIN’-GmbH, histopathological examination of ‘Negri’ corpuscles and the mouse inoculation test (MIT). A large-scale rabies survey started in 1997 involving different geographical regions and focussing mainly on red foxes and other wild terrestrial carnivores; dogs, cats and bats are included as well. During the period 1997–2009, 1 220 animals, comprising 681 wild carnivores, 409 bats and 130 domestic animals were destroyed.

Unfortunately, Albania has not yet been able to introduce mass wildlife immunisation with oral vaccines due to lack of well-developed vaccination strategies based on prior ecological studies of target animals, appropriate planning, trained personnel and of course, adequate funding.

**CONCLUSIONS**

Government expenditures on agriculture have decreased in recent years, with the bulk of Ministry of Agriculture, Food and Consumer
Protection (MAFCP) expenditures (48% of the budget in 2005) going to investments in irrigation and drainage infrastructure. However, the ‘Agricultural and Food Safety Inspections and Services and Consumer Protection’ Programme (the third largest in MAFCP with 15% of the MAFCP budget), is expected to see significant increases in the coming years to strengthen inspection services.

Given the limited absorptive capacity of relevant institutions, additional funding should be selectively applied and well prioritised. Establishing a national food safety system consistent with the EU Acquis will require increased levels of public expenditures on food safety, veterinary and phytosanitary activities. Establishing a national food safety system consistent with the Acquis is a priority and adequate financial resources should be allocated to support the creation of institutions and systems and to upgrade skills.

The challenge faced by a reliable National Animal Health Programme, is to sustain livestock production, including social needs, in the agricultural community, through modern economic approaches. The NAHP can be the core for this type of sustainability due to the trust of the agricultural community in the veterinary input. However, the NAHP should use police authority to implement necessary quarantine measures in case of transboundary diseases.

The overall capacity of the country’s laboratories is deficient and should be further developed in order to provide food testing and analytical services that meet international standards and requirements. Although there are 36 laboratories throughout the country, most have inadequate or outdated equipment and infrastructure, a shortage of competent analytical and managerial staff, no official working methods or procedures or business plans, and poorly developed systems for recording test results, reporting, and information management. In some cases, laboratories have obtained sophisticated equipment under international projects, but analysts and technicians lack the necessary skills to operate and maintain them. Other challenges to be overcome include shortages in the power supply and lack of funds to meet operating costs. Many laboratories face difficulties in obtaining essential supplies of materials, reagents and services (disposable materials, reagents, gases, calibration and maintenance services), and lack access to technical support for calibration and reference testing. A further problem is the loss of qualified staff, including individuals trained by donor projects, either through dismissal or transitioning to other jobs.

REFERENCES


A symposium on 'Sustainable Improvement of Animal Production and Health' that was organised by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture in cooperation with the Animal Production and Health Division of the Food and Agriculture Organization of the United Nations addressed the animal husbandry and public health issues that threaten global food security and safety. The growing world population is vulnerable to limitations in the production of agricultural products and any change, be it climatic realities and/or variations or civil strife upset the delicate balance of providing affordable food for all. It is alarming that the world's poorest people, some one billion living mostly in Africa and Asia, depend on livestock for their day-to-day livelihood. To reduce poverty, fight hunger and ensure global food security, there is an urgent need to increase livestock production in sustainable ways.

This publication provides invaluable information not only on how nuclear and related techniques can be used to support sustainable livestock production systems, but also about the constraints and opportunities for using these techniques in developing countries; it attempts to identify specific research needs and gaps and new options for using these techniques for solving established and emerging problems. As such, it is hoped that the information presented and suggestions made will provide valuable guidance to scientists in both the public and private sectors as well as to government and institutional policy and decision makers.