

## RECOMMENDATIONS ON SWINE INFLUENZA VACCINES AND VACCINATION STRATEGIES

**K. Van Reeth and M. Pensaert**

*Laboratory of Virology, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium*

### Introduction

Vaccination remains the primary means of preventing SIV in pigs. Commercial, inactivated SIV vaccines have been on the market in Europe since the early 1980s, and they contain both of the subtypes that were prevalent at that time, H1N1 and H3N2. However, the recent changes in the epidemiology of SIV have raised many questions with regard to the efficacy of these vaccines against the current strains. In this paper, we will first present basic information on the immune response to SIV infection and vaccination, because this is necessary to understand how vaccines work and what one can achieve with vaccination. Thereafter, we will review a few questions that are frequently asked by vaccine manufacturers and swine practitioners. The viewpoints presented are based on data from the EC concerted action «European Surveillance Network for Influenza in Pigs», as well as on pig experiments performed in the authors' laboratory.

### The immune response to an infection with SIV

An infection with SIV induces a rapid and efficient immune response, which results in complete elimination of the virus within a week and a very solid protection against reinfection. The specific immune response to SIV includes the production of antibodies in the circulation and at the mucosae of the respiratory tract, as well as a cell-mediated immune response. Antibodies develop to the haemagglutinin (HA), neuraminidase, matrix and nucleoprotein proteins. However, only antibodies to the globular head region of the HA, which are detectable by virus neutralization (VN) or haemagglutination inhibition (HI) assays, can neutralize the virus and thus prevent an infection. These HA-specific neutralizing antibodies are highly efficient mediators of protection against reinfection with a similar virus strain, but they will not protect against strains belonging to another SIV subtype. In recent infection experiments, however, we have demonstrated some type of cross-protection between the current European H1N1, H3N2 and H1N2 SIV subtypes in the absence of cross-reactive HI antibodies (1). In contrast to pigs that had been previously infected with either H1N1 or H3N2, pigs with infection-immunity to both subtypes showed a solid protection against H1N2 infection. Still, these pigs only had HI antibodies to H1N1 and H3N2 at the time of H1N2 challenge. This suggests that some of the other immune mechanisms, which are generally less effective but more cross-reactive between influenza viruses, contribute to this cross-subtype protection. The cell-mediated immune response, for example, which is largely directed against the conserved internal viral proteins, is probably involved.

### The current SIV vaccines and the immune response to vaccination

SIV vaccines are based on whole, inactivated influenza virus, or on highly purified disrupted virus particles («split» vaccines) and an oil adjuvant. Most of the current vaccines still contain the older human New Jersey/76 (H1N1) and Port Chalmers/73 (H3N2) strains, but no

H1N2 component. The primary vaccination should consist of two intramuscular injections 3 to 4 weeks apart, and bi-annual booster vaccinations are recommended for sows.

As for other inactivated vaccines, the immune response to SIV vaccines differs from that following replication of infectious virus in the host. SIV vaccines mainly induce circulating antibodies to the HA of the vaccine strains, while mucosal or cellular immune responses are barely stimulated (2). The presence of high titres of neutralizing antibodies in the serum, which can reach the lungs by diffusion, is sufficient to block or significantly reduce SIV replication in the lungs in case of an infection, and to prevent disease. Such a reduction of lung virus titres appears to result in a reduced production of proinflammatory cytokines in the lungs, which are thought to be essential mediators of the typical SIV symptoms (3). Experimental data have clearly shown that a minimal reduction of virus replication in the lungs will strongly reduce cytokine levels and thus protect against disease.

Because protection following vaccination is almost entirely dependent on HI antibodies in the circulation, antibody titres to the infecting strain and protection are tightly correlated. In vaccination-challenge studies by the authors, all pigs with HI antibody titres >160 were completely protected against virus replication in the lungs and disease (4). Pigs with lower antibody titres showed a significant reduction of lung virus titres when compared to unvaccinated controls, and they were still completely protected from disease. However, we used a very severe challenge method in these studies ( $10^{7.5}$  EID<sub>50</sub> virus intratracheally), and antibody titres  $\geq 160$  may be effective against challenge with a lower virus dose or under field conditions. On the other hand, protection induced by vaccination is somewhat more specific than that after infection and this issue is further discussed in the questions below.

### Is there a need to update H1N1 and H3N2 vaccine strains?

It is generally accepted that antigenic drift of circulating influenza virus strains in comparison with vaccine strains may render vaccines less effective, and human or equine influenza vaccine strains are therefore regularly updated. Replacement of the New Jersey/76 (H1N1) and Port Chalmers/73 (H3N2) strains in SIV vaccines has also been considered, based on reports of antigenic drift in European H1N1 and H3N2 SIVs during the late 1990s (5,6). On the other hand, antigenic analyses performed during the ESNIP concerted action have clearly shown that antigenic drift in swine influenza viruses is minimal when compared to that occurring with human influenza viruses over a 20-year period. Most important, commercial New Jersey/76 (H1N1) and Port Chalmers/73 (H3N2) based vaccines were still very efficacious against more recent strains in pig experiments. In studies by the authors, a double vaccination with such a vaccine conferred excellent protection against a severe intratracheal challenge with H1N1 or H3N2 viruses isolated in Belgium in '98 (4,7). Despite the antigenic differences between vaccine and

challenge strains, the commercial vaccine still induced high antibody titres to the field strains. Challenge virus replication in the lungs was undetectable or strongly reduced and there was no disease. Similar results were obtained in challenge studies with an H3N2 challenge virus isolated in The Netherlands in 1996 (2). There are thus no scientific arguments to update the H1N1 or H3N2 vaccine strains.

#### **Do the current vaccines protect against H1N2?**

Under experimental conditions, the commercial SIV vaccine that protected so efficiently against recent H1N1 and H3N2 strains did not protect against challenge with the H1N2 subtype (1). The vaccine induced little if any HI antibody to H1N2, and it could not prevent H1N2 virus replication or disease upon challenge. In contrast, the addition of an experimentally prepared H1N2 component to the vaccine conferred significant protection from H1N2 infection and disease. It is still unknown how the absence of an H1N2 component in the vaccine affects vaccine performance in the field, but the H1N2 subtype has clearly become widespread throughout Europe. Therefore, the inclusion of an H1N2 strain in SIV vaccines must be considered.

The failure of (H1N1+H3N2) vaccines to protect against H1N2 also points towards a role of cellular and/or local immunity in the protection to H1N2 in (H1N1+H3N2) infection-immune pigs.

#### **How much antigenic drift is needed before vaccine strains become obsolete?**

This is not exactly known. The vaccine strains must show some antigenic overlap with the infecting strains to be protective, but antigenic (cross HI tests) and genetic analyses are not the most accurate predictors of vaccine strain performance. In fact, many of the antigenic and genetic variations found within H1N1 and H3N2 SIV subtypes appear to have little impact on vaccine efficacy in the pig, as illustrated by the experiments mentioned higher (2,4,7). On the other hand, dramatic antigenic differences, such as that between the current H1N1 vaccine strain and the circulating H1N2 strains, will compromise vaccine efficacy. In genetic analyses, we found as much as 99 amino acid changes between both strains, and 39 of them were located in antigenic sites. This compares with 28 amino acid differences in five antigenic sites between the H1N1 vaccine and challenge strains used in pig experiments (1, 7). Unfortunately, genetic analyses of influenza viruses are rarely combined with *in vivo* vaccination-challenge studies and there is still a significant lack of knowledge concerning the impact of genetic drift on vaccine efficacy. Another important issue is that factors other than the nature of the vaccine strains, such as the antigenic dose and adjuvant, can also have a dramatic effect on vaccine efficacy. Therefore, challenge tests in pigs remain essential to evaluate vaccine efficacy.

#### **How efficient is vaccination in the field?**

There are few published data on SIV vaccine efficacy in the field. While experimental studies generally use SIV seronegative pigs and an optimal time interval between vaccination and infection, this may be different in the field. Maternal antibodies, for example, frequently interfere with

effective vaccination of feeder pigs. Furthermore, the cost-benefit of SIV vaccination is often questioned. Though an acute SIV outbreak can cause serious disease and weight loss in fatteners, recovery is rapid in uncomplicated cases and pigs may catch up on their weight within 2-3 weeks.

#### **Should we vaccinate sows or fattening pigs?**

Serological data indicate that vaccination of the sows is likely to be beneficial for both the sow and her offspring. Indeed, significantly higher H1N1 and H3N2 antibody titres are seen in vaccinated (frequently 1:160-1:640 or greater) than in unvaccinated sows. This results in high and long-lasting maternal SIV antibody levels in the piglets from vaccinated sows. In a study by Thacker (2000), SIV passive antibody levels dropped below 1:40 by 6 weeks of age in nearly all pigs from unvaccinated sows, which had only low HI titres. In contrast, antibody titres in pigs from vaccinated sows were frequently detectable until 16 weeks of age. As mentioned previously (see paper on SIV serology), the high antibody titres in vaccinated sows may have been stimulated by previous infections with SIV. In experimental studies, SIV vaccination of infection-immune pigs caused a dramatic increase of HI and VN antibody titres to all subtypes with which the pigs had previously been infected. It is unlikely, therefore, to encounter problems with SIV in sows that have been routinely vaccinated or in their newborn pigs.

Vaccination of feeder pigs is less commonly performed. This strategy may be recommended in herds where influenza is a problem in growers or finishers. One difficulty is that even very low levels of residual maternal antibodies can interfere with vaccination of young pigs. Vaccination of feeder pigs is therefore difficult to combine with vaccination of sows, since prolonged passive immunity may interfere with effective vaccination of piglets.

**Conclusion:** Of all vaccines against respiratory viruses of pigs, SIV vaccines are among the most effective. One weakness of the current vaccines is that they do not protect against the novel H1N2 subtype under experimental conditions. Still, the available field data suggest that vaccination of sows is highly efficient in controlling disease in suckling pigs and may protect pigs throughout the nursery phase.

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## THE MEASUREMENT OF TOTAL VOLATILE NITROGEN (TVN) IN QUALITY CONTROL OF SOME BONY FISH IN THE RETAIL MARKETS OF THE CITY OF SHAHREKORD, IRAN.

**Amir Shakerian<sup>1\*</sup>, N. Rokni, A.Ziaii, M. Boniadian**  
*T-ISLAMIC AZAD UNIVERSITY OF SHAHREKORD, IRAN.*

### Abstract

In the spring and summer of 2003, the Total 90 samples from three species of fishes, which are available in south sea and are called: (*Chirocentrus dorab*, *Teuthis siganus* and *Hilsa kanagurta*) were collected, from retail markets. The samples were examined by kjeldahl method for measurement of TVN in the meats. The results showed that in 5.55% of samples, TVN were more than normal rate. The mean value of TVN in *Chirocentrus dorab*, *Teuthis siganus* And *Hilsa kanagurta* were 20.3, 19.37 and 17.69 mg/100 g of meat respectively.

The rate of TVN in the central districts of the city of Shahrekord was lower than out skirts. And it is because of better supply, keeping of the fish and specially the better quality of non-frozen fishes.

Key words: Total Volatile Nitrogen (TVN), Quality control, and Bony fish, Retail markets.

### Introduction

With regards to the importance of fish and fish products as an important available resources of animal proteins and with attention to their rapid spoilage, it is necessary to open a new window in rapid and economic control of these products. Therefore we have carried out a study on 90 samples of bony fish in the retail markets sale in Shahrekord, IRAN, in 2003, with macro kjeldal method for determination of Total Volatile Nitrogen (TVN).

### Material and methods

In the spring and summer of 2003, the Total 90 samples from three species of fishes, which are available in south

sea and are called: (*Chirocentrus dorab*, *Teuthis siganus* and *Hilsa kanagurta*) were collected, from retail markets. The samples were examined by kjeldahl method by A.O.A.C methods for measurement of TVN in the meat. 10 g from meat bony fish was obtain and to place in kjeldahl distillation system, then Volatile Nitrogen in glass balloon (to contain Boric acid 2%, methyl red, Bromocresol green), was collected and to titration by sulfuric acid (0.1 N) for measurement of TVN by mg / 100g of fish meat (1,2).

### Results

Out of 90 meat bony fish samples, 5(5.55%) of samples, TVN were more than normal rates (25mg/100g meat).

### Discussion

In comparison with previous study in Iran, for examples in Tehran, IRAN in 1999, in 85.7% of samples, TVN were more than normal rate(1). So in other study in Tehran, in 2000, in 3.4% of samples TVN were more than normal rate (1). The rate of TVN in the central districts of the city of Shahrekord was lower than out skirts. And it is because of better supply, keeping of the fish and specially the better quality of non-frozen fishes.

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Table- 1) Characteristic of three species of bony fishes to measurement of TVN, in Shahrekord , IRAN(2003).

Species of bony fishes	Mean value	S.E	S.D	Max.	Min.
<i>Chirocentrus dorab</i>	20.3	0.55	3	28	15.4
<i>Teuthis siganus</i>	19.37	0.66	3.615	28	15.4
<i>Hilsa kanagurta</i>	17.69	.045	2.45	23.8	15.4



## THE MATRESA PROJECT – TREATMENT STRATEGIES FOR LIVESTOCK MANURE FOR SUSTAINABLE LIVESTOCK AGRICULTURE

C H Burton and C Turner

*Silsoe Research Institute, Silsoe, Bedford. UK*

The EU-funded MATRESA project was concluded in 2003 with the publication of a detailed reference book\* that sets out a thorough review of the management and treatment of agricultural wastes across Europe. The objective was to raise awareness among European agriculturalists (including farmers, advisors and local authorities) of the current research and technology available within Europe to facilitate better management of livestock wastes to (a) minimize environmental and health hazards and, (b), gain the maximum benefit. Information was drawn from the contributions of project partners representing 24 countries - engineers, agronomists, vets and scientists were chosen for their involvement in national and European programmes.

A central finding of this review was that good management of livestock wastes (eg, the collection, storage, mixing, pumping and spreading of livestock manures) following existing guidelines can alleviate problems in *some* circumstances, but it is rarely a complete solution. Some livestock farms simply lack enough suitable land to safely receive the manures produced. The application of excessive quantities of livestock manure (and/or mismanagement) is already leading to a range of pollution problems. These include water contamination (by nitrates, phosphates and organic matter) air emissions (including ammonia, nitrous oxide and methane) and soil residues (including phosphates and heavy metals). Poor manure handling can also lead to disease risks to farm animals, the general public and food production in general.

### **Sustainable agriculture in Europe**

Today, agricultural production systems in Western Europe are highly developed with individual farms tending to specialize; resources are used very efficiently and output is high. Nonetheless, as a consequence, local and regional surpluses are generated; supplies and products are transported over increasing distances. For the manures and effluent produced, local land disposal remains the main option but they often become regarded as waste streams and treated accordingly. However, the more sustainable situation essentially involves greater recycling and reduced losses to the environment; input of inorganic fertilizer can then be reduced as a result. In order to reach such a situation, changes in approach will be needed from those in the agricultural business as well as from the authorities and the public in general.

### **Manure and effluent management**

#### *Water management issues*

One of the key difficulties with handling many liquid animal manures lies with their relatively low concentrations of dry matter. For some dairy waste-waters (or dirty waters) this value can be well below 10 kg/m<sup>3</sup>. The implications are threefold; (i) there is a need for larger storage capacity, (ii) the application to meet crop requirement is more difficult and (iii) large quantities of water are being used implying

increased transportation. Reduction of manure volume by using less water thus has clear benefits and there are various guidelines for efficient water use on a farm. There has been some research on re-using partly treated slurry for flushing channels in buildings. This has the benefit of both reducing water requirement and increasing the solids concentration in the slurry. The treatment implied may be simply a physical clarification process or it may include some biological activity as well to degrade the dissolved organic matter. The limitation of this strategy lies with the cost and efficiency of the treatment process involved balanced against the penalties of the alternatives; eg using more water and needing to deal with larger volumes.

#### *Transportation of livestock manures*

Moving manures from region to region represents a seemingly simple solution to the environmental problems of those areas with excess nutrients. However, this approach is fraught with problems based on the scale of the operation, nutrient monitoring and in some cases, disease risks. The problem is mostly attributable to the volume of liquid slurry; in many cases, the solid wastes (eg, the farmyard manure) could be beneficially used without problem on the farm or locally. However, slurries often contain more than 95% water hence pre-concentration is important if the exercise is not to become one of moving water. Such an approach will require low cost concentration systems if it is to be viable; the implication is some form of physical process with a very dilute waste water being irrigated locally.

The relatively low concentration of dry matter in most slurries does enable transport by pipeline which may be a more practical option for shorter distances. Some pre-screening is necessary to remove suspended matter that may lead to blockage. Otherwise, the issue comes down to the question of investment in pipeline systems as much of the technology already exists. Concern over disease spread may yet be the greatest hurdle to large scale redistribution of livestock slurries.

### **Treatment systems in agriculture**

It is unlikely that complete abatement of pollution and the other problems associated with livestock manure can be achieved by improved farming practice alone. In some situations further measures including treatment will form part of the solution. Even where there is adequate land available and a good nutrient balance, some form of treatment may still be appropriate e.g., for odour abatement or to minimize disease risks. These can be physical, biological, chemical or a combination of all these processes.

Treatment has a clear role in the overall management package, but only some of systems emerging are both practicable and effective at the farm level. The broad theme behind good manure management is proposed as one based on aiming for a more balanced farming system to avoid the

release of excess nutrients into the environment. This implies greater targeting of nutrients in manures to meet the crop need and a subsequent reduction in the applied level of inorganic fertilizers. However, improved monitoring in the application of the nutrients in raw and treated manures is necessary to reduce the uncertainty on the subsequent interaction with the soil and crop uptake. Aerobic treatment can remove unwanted nutrients or stabilize them to enhance plant utilization; it is also effective in odour abatement. Information is lacking though to enable an objective comparison and evaluation of such processes and although effective, the general cost is still too high for many farms. Reducing the manure burden of a farm lacking enough land implies the export of surpluses. Even with improved transportation systems, some pre-concentration is desirable.

The implied volume reduction can have an additional benefit in enabling improved water use in and around the farm. Conversion of solid manure and livestock slurries to a range of saleable products is an attractive option but quality and consistency are important. This may involve the co-processing with other organic wastes to gain a balanced blend. Separate from farming, manure surpluses may yet be a resource for industry in the future owing to the wide range of chemicals it contains.

#### *Process equipment design and verification*

There is a wide range of technology and related machinery available now for the use of processing the various livestock manures. Much of this originates from designs used in other industries especially sewage treatment and water supply. However, the satisfactory application to the much stronger effluents from agriculture does not necessarily follow; the objectives for treatment are not necessarily the same and available funds are usually much less. A key problem lies with a systematic evaluation of the individual machine or complete process; what is it achieving, what are the costs and how does it compare with the alternatives? The response to this is in part a matter of policy making, ie, setting specific environmental standards, but this is not so simple when it comes down to objectively scoring a piece of equipment. A typical claim that a process "reduces water pollution" is obviously vague and clearly much will depend on other agricultural factors. However, a more precise standard can often be identified such as aerator performance in kg oxygen dissolved per kWh of electricity consumed. Likewise, a process may be rated in terms of the percentage of nitrogen removed (or conserved as the organic form) - the full benefit of the process will still depend on other agricultural factors (*eg* spreading method and timing) but they will be the same for any process chosen.

#### **Conclusion - are there any "best" options?**

One of the first issues to arise from the workshop meetings that gave rise to this publication is the wide range of farming scenarios across Europe. Factors such as farm size, local geography and land type, climate and production method all give rise to farms with highly individual features.

It is not surprising then that there are no universal solutions to the manure problems experienced on livestock farms. Rather, the many methods are likely to be as highly individual as the farms themselves. However, the situation can be rationalized to some extent by the grouping of farms according to farm type and dominant manure problem(s) - each such group would then suit a manure management strategy and for each there may be one (or more) best options.

A second general theme to arise from this collaboration was that treatment should not be as the first choice in dealing with the perceived problems on a farm. Indeed, owing to the relatively high costs often involved, treatment should only be considered when existing methods of good manure management have been implemented and found to be inadequate. However, when a problem persists despite running a good farm operation and action is required, then the treatment option is necessary.

The key message is one of correctly identifying the problem and setting out an effective and verifiable strategy to deal with it. This involves being specific on what is required of the waste management plan thus enabling the selection of effective technology that meets the requirements.

\* BURTON, C.H.; TURNER, C. (editors) (2003) *Manure management - treatment strategies for sustainable agriculture; second edition* Silsoe Research Institute, Wrest Park, Silsoe, Bedford, UK. 490 pages.

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