The application of macrolides as a control aid for PRRS

by Wouter Depondt, DVM, global product manager, Huvepharma, Belgium.

Macrolides are a group of antibiotics whose activity stems from the presence of the macrolide ring to which one or more deoxy sugars, usually cladinose and desosamine, may be attached.

Macrolides tend to accumulate within leukocytes and are transported into the site of infection and are used to treat respiratory infections caused by Mycoplasma spp and Gram negative bacteria such as Pasteurella multocida and Actinobacillus pleuropneumoniae.

In addition to the typical antibiotic effect, some macrolides also have three other properties; anti-inflammatory, immunomodulatory and anti-viral activity. These actions of macrolides encouraged a number of researchers and practitioners to explore the application of macrolides for viral infections.

In veterinary medicine, field reports and publications describe the antiviral activity against Porcine Reproductive and Respiratory Syndrome virus (PRRSV) of tilmicosin, the active of Huvepharma's brand Tilmovet.

Porcine reproductive and respiratory syndrome (PRRS) is an important disease of pigs defined by severe respiratory disorders in piglets and widespread abortions in gestating sows. This article describes three trials, to investigate the anti-viral activity of Tilmovet.

Weaned piglets suffering from conjunctivitis and sneezing.

First the effect of this antibiotic was evaluated in-vitro by Frydas in 2012. Porcine alveolar macrophages (PAMs) were pre-treated with tilmicosin (Tilmovet) at 0.1mg/ml and 0.01mg/ml for six hours, then seeded in 24 well plates and inoculated for one hour with 250µl of $10^{6.3}TCID_{50}$/ml European type 1 strain.

Subsequently a medium with Tilmovet was added at the same concentration as the pre-treatment and the cells were incubated for six hours. A non-treated control group was also included. The percentage of infected PAMs was evaluated by subjecting them to PRRSV specific immunoperoxidase staining.

Tilmovet suppressed the replication of PRRSV at concentrations 0.1mg Tilmovet/ml and 0.01mg Tilmovet/ml by 48% and 57% respectively (P<0.001) (see Fig. 1).

After confirming the in-vitro activity of this antibiotic for PRRSV, the in-vivo efficacy was tested. Forty four-week-old weaned piglets were randomly chosen from a PRRSV-contaminated farrow-to-finish herd.

The piglets were divided equally into two groups and housed in the same pen but separated into individual spaces.

Tilmicosin (Tilmovet 20% premix, 2kg/ton) was administered to the four-week-old piglets for three weeks (treated group). The control group did not receive any treatment (untreated group). Blood samples were collected from each animal every two weeks. The PRRSV viral load was quantified by ZNA probe-based real-time PCR. Student's t-test was used to compare the viral loads.

Mortality rate and daily weight gain were recorded to evaluate clinical signs. At the beginning of the trial and two weeks later no differences were observed in viral load. After eight week of age (four weeks after starting the trial) a ten-fold reduction in viral load was observed (Table 1).

The treated animals exhibited a drop in mortality rate of 20% (5% versus 25%) and an increase of average daily weight gain of 0.03kg (0.45kg versus 0.48kg) during the period of the study.

The third trial was a field trial performed at a Belgian closed 500 sow farrow-to-finish farm (high biosecurity level: five week batch-farrowing and all-in-all-out) with symptoms indicative for PRRSV, such as mummification, stillbirth, late-term abortions, weak-born pigs and PHS (Periparturient Hypogalactia Syndrome) in the breeding herd.

Also, a high mortality rate was seen in the nursery caused by respiratory disease and from secondary bacterial infections. The sows and gilts were vaccinated at day 60 of pregnancy with an attenuated PRRSV vaccine (European strain) and an inactivated atrophic rhinitis vaccine. The gilts were additionally vaccinated with a one-shot Mycoplasma hyopneumoniae vaccine, 4-6 weeks before movement to the breeding department. The piglets did not receive any vaccinations.

	Group		
	Untreated	**Treated**	**P value**
Number of tested samples	93	96	-
Number of positive pigs (%)	31 (33.3)	30 (31.3)	0.759
Mean viral load ± SD[a]	**3.38 ± 1.01****	**2.58 ± 0.60**	**0.0003**
Median[a]	3.17	2.50	-
Range[a]	1.96-5.19	1.25-3.73	-

[a]Log_{10} copy number per microlitre in serum, ** Statistical significance (P<0.01)

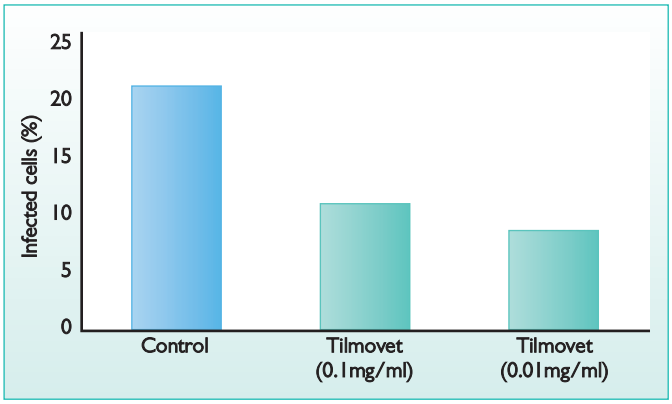
Table 1. Average PRRSV load of all serum collected from the treated and untreated groups.

First a serological survey was performed in December 2012. Blood samples were taken from 10 gilts and 10 sows. Additionally, 30 blood samples were taken in a way that all ages of weaned piglets and fatteners were represented by six samples per age category. The blood samples were sent to Dialab (Belgium) for determining PRRSV ELISA S/P ratios (PRRS herdcheck X3).

High and variable PRRSV S/P ratios (Fig. 2) between 0 and 4.11 were observed in the breeders and in the progeny for all ages.

Continued on page 9

Fig. 1. Effect of Tilmovet on PRRSV replication in PAMs (P< 0.001).



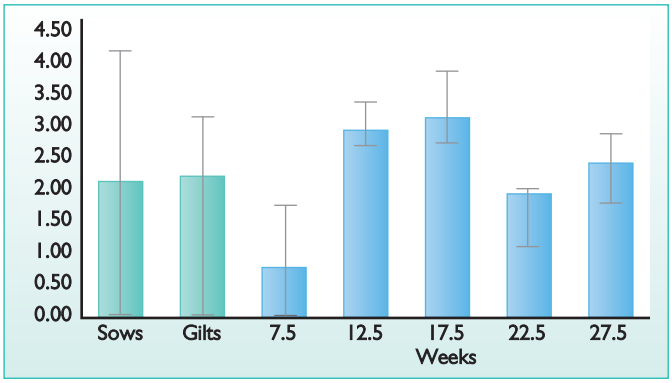


Fig. 2. PRRS S/P ratios from the blood samples taken in December 2012.

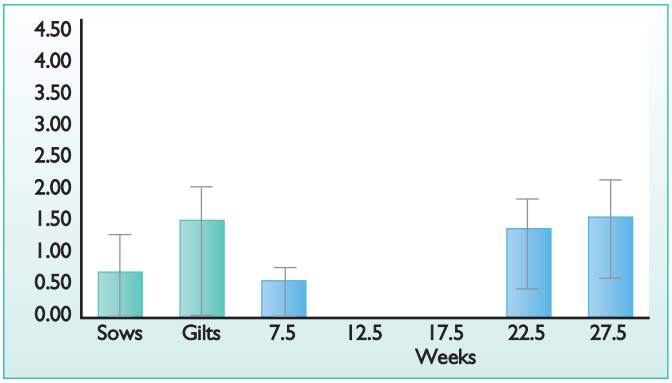


Fig. 3. PRRSV S/P ratios from the blood samples taken in July 2013.

Continued from page 7

A treatment was implemented with Tilmovet at the end December 2012. The treatment scheme was based on previously published treatment schemes with tilmicosin for control of PRRSV. Gilts and weaned piglets are often considered as a source of infection. For this reason all gilts were treated with 10mg tilmicosin per kilogram bodyweight as Tilmovet 100 oral granules for 14 days after movement from the quarantine to the breeding house.

All weaned piglets were treated with 16mg tilmicosin per kilogram bodyweight as Tilmovet 250 premix for seven days after weaning. Most of the clinical symptoms, such as early farrowing and PHS, occurred at the end of gestation and it has been described that a higher number of congenital infections and a higher number of infected foetuses/newborn piglets are observed upon inoculation of sows at late gestation.

Moreover the shift from cellular-immunity toward humoral immunity in late gestation might enhance clinical outbreaks of PRRSV during this period. It has also been shown that PRRSV is shed in the milk and colostrum of infected dams.

Tilmicosin concentrating in colostrum and milk might help to lower replication and infection of PRRSV in piglets during the first weeks after farrowing.

This is why Tilmovet was administered to the sows at 10mg tilmicosin per kilogram bodyweight per day as Tilmovet premix in the feed seven days before until seven days after farrowing.

At the beginning of July 2013 blood samples were taken from gilts, sows and the progeny for determination of PRSSV S/P ratios. Also the breeding performance was evaluated.

Six months after implementing the treatment scheme, a drop in S/P ratio was observed in all age groups. The mean S/ P ratio in the breeding herd was 0.64 and ranged between 0 and 1.25. In the piglets aged 7.5 weeks, two were found slightly positive, most likely reflecting the maternal antibodies.

The fatteners stayed serologically negative until the age of 22.5 weeks (Fig. 3).

In addition to the blood samples, breeding performance was evaluated by comparing the results from 2012, before implementation of the Tilmovet treatment scheme, with the results from January 2013 until September 2013, during the Tilmovet treatment scheme.

The average of weaned piglets per year increased from 29.52 to 32.58. This was mainly due to the number of weaned piglets rising from 12.71 to 13.73 (Table 3).

Veterinarians are often confronted with recurrent outbreaks of PRRSV. This field case describes an alternative way to control PRRSV and has been reproduced several times in the meantime.

It was not possible to completely eradicate PRRSV from the farm, but this treatment scheme allowed the stabilisation of PRRSV in the breeding herd and to control the clinical symptoms of PRRSV, resulting in better breeding performance.

The treatment also resulted in less vertical transmission of PRRSV to the progeny and consequently a lower and later infection pressure.

Concurrent infection with PRRSV and, for example, Mycoplasma hyopneumoniae vaccination might lead to lower efficacy of the vaccination. Moreover, it appears that PRRSV infection increases the severity of M. hyopneumoniae-induced pneumonia early in the course of the disease. This is why creating a PRRSV stable sow herd might help to enhance the efficacy of vaccinations administered to the piglets and to decrease the clinical symptoms of PRDC (Porcine Respiratory Disease Complex). Due to practical reasons the piglets were only treated with Tilmovet for seven days. Further research is needed to elucidate the effect of a longer treatment after weaning. ■

Tilmovet is a registered trademark of Huvepharma.
References are available on request.

Table 2. Treatment scheme applied in field trial.

	Period (days)	Timing	Dosage (mg/kg)
Gilts	21	Entering breeding herd	10
Sows	14	Seven days before farrowing until seven days after	10
Piglets	7	After weaning	16

Table 3. Evolution of breeding performances.

	2012	2013
Cycles/year	2.32	2.38
Live born	14.52	15.26
Weaned	12.71	13.73
Mortality (%)	11.38	10.19
Weaning age	23.74	22.74
Weaned piglets	**29.52**	**32.58**