

tracheal swabs were collected at each sampling. The serological screening was performed using Rapid Slide Agglutination (RSA) according to the OIE protocol and indirect ELISA (IDEXX) according to the manufacturer's instructions. The molecular diagnosis was performed using a commercial kit of a duplex real time PCR (Lifetechnology). The results revealed that One day old chicks were negative to MS by RSA and PCR, however they have a variable stock of maternal antibodies (Ig Y) detected by iELISA. The seroprevalence found by RSA is variable and increase with the age (8th week: 55%, 15th week: 91%, 32th and 58th week: 100%), the same profile was traced by PCR (8th week: 36%, 16th week: 64%, 32th week: 82%, 58th week: 100%), however, all farms were positive by iELISA, from the first day to 58th weeks. These results show that MS infections are very frequent and very widespread among in poultry breeder flocks, and showed a perfect agreement between serological tests and Real time PCR starting from 32th week of age.

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Growth, re-isolation and titration of *M. gallisepticum* ts-11 and *M. synoviae* MSH vaccine strains – the need for an appropriately validated media.

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Experience with ts-11 and also MSH in laboratories (using media made in house or purchased) around the world suggests that these strains are unique requiring quality control of media to make sure that these strains can grow optimally in media rather than the media being quality controlled on other strains. The best way to do this is to have reference preparations of ts-11 and MSH to control media. The easiest way to do this is to get a large number of vaccine vials of the same serial stored at -70C. If a media cannot get the reference preparation to the expected titre then titration of these vaccines strains with that media is invalid. This effect of media can vary from batch to batch and has been as large as a 6 log₁₀ depression of growth. There is some suggestion that this effect is labile (due to heat and/or time since preparation) and perhaps from the yeast extract. This media quality problem has caused some problems. Government laboratories testing potency can get media from our manufacturing facility to titrate vaccine or develop their own media. Re-isolation studies have been severely biased by media quality making conclusions about strain displacement unreliable. Conversely it is probably not a problem in antibiotic sensitivity testing and perhaps the vaccine strains can be adapted to other media formulations but whether this would also change the properties under investigation is not known. Technically, the gold standard for titration is CFU- this allows an error estimate to be made from the replicates. CCU calculations have been traditionally used in mycoplasma quantification of vaccines but they have an intrinsic problem with variable experimental error because these are technically easier and less susceptible to contamination during the later period of incubation. Automation of titration with dilution being done on plates is incompatible with an assumption in calculation of MPN (Poisson distribution) – that the samples are taken independent of each other. The dilution series needs to be done off the plate. At least with MPN there are usually 8 to 12 replicates per dilution. Some people have also used Spearman Karber calculations but with no replicates. Only one possible significant for titre is possible if it is done without replicates. In CCU determinations how do we read titration end points with only a partial colour change occurs? Especially in laboratory temperature sensitivity tests. (These need to be done in incubators with temperature control in the non-permissive incubator being ±0.1C). Also descriptions of media preparations often are not clear that glucose should not be autoclaved with phosphates.

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***Mycoplasma synoviae* seroprevalence in Dutch poultry: an update**

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Until the year 2012, the control and eradication of *M. synoviae* in our country was voluntary and has been limited to grandparent stock. The growing clinical and economic relevance of *M. synoviae* has prompted the Dutch poultry industry to start a nationwide control and eradication programme for this mycoplasma species. A study performed in 2005-2006 describing for the first time the *M. synoviae* seroprevalence in different categories of commercial poultry in the Netherlands showed a high *M. synoviae* seroprevalence,

especially in rearing layers and layers where it was 100% and 90% respectively. In other categories of poultry it was significantly lower. The awareness of the importance of *M. synoviae* was further increased due to disease outbreaks with arthropathogenic *M. synoviae* strains and strains including EAA. In 2013, the Dutch Commodity Board for Poultry and Eggs implemented a compulsory *M. synoviae* monitoring programme (Besluit onderzoek *Mycoplasma gallisepticum*, *M. synoviae* en *M. meleagridis* 2012) aiming at identifying *M. synoviae* infected flocks in order to control and eradicate *M. synoviae*. In 2013, seromonitoring was done using the *M. synoviae*-RPA test and ELISA test. The sampling frequency and sample sizes for the seromonitoring of *M. synoviae* are equal to those used for the control and eradication of *M. gallisepticum*. However, in 2014 flocks vaccinated against *M. synoviae* will be monitored using a PCR test able to differentiate *M. synoviae* vaccine from field strains. Control measures consist of the voluntary slaughter of *M. synoviae* infected grandparent stock, partial channelling to maintain separation of infected flocks and eggs from those with infection, and improvement of biosecurity. The results of the *M. synoviae* seromonitoring data obtained in the third quarter of 2013 indicate that the percentage of *M. synoviae* infected farms has significantly decreased in meat grandparent stock (2005 = 10% and 2013 = 0% with a 95% C.I. = calculated prevalence), in breeder layer stock (2005 = 25% with a 95% C.I. of 19 to 31 and 2013 = 15% with a 95% C.I. = calculated prevalence), turkey stock (2005 = 16% with a 95% C.I. of 10 to 22 and 2013 = 3% with a 95% C.I. of 0 to 6) and rearing layer stock (2005 = 69% with a 95% C.I. of 67 to 70 and 2013 = 16% with a 95% C.I. of 13 to 19). However, in broiler breeders and layers the percentage of *M. synoviae* infected farms did not decrease significantly (breeders in 2005 = 35% with a 95% C.I. of 35 to 44 and in 2013 = 53% with a 95% C.I. = calculated prevalence; layers in 2005 = 73% with a 95% C.I. of 67 to 80 and in 2013 = 74% with a 95% C.I. of 70 to 79). This was explained by the lack of partial channelling as a control measure for *M. synoviae*. The declining seroprevalence of *M. synoviae* in meat grandparent, breeder layer, meat turkeys and rearing layers indicates that control and eradication of *M. synoviae* is feasible with the measures taken.

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Study of infections by *Ureaplasma parvum*, *U. urealyticum* and *Mycoplasma hominis* in infertile women, 2010-2012

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At the present time *Ureaplasma parvum* (Up), *U. urealyticum* (Uu) and *Mycoplasma hominis* (Mh) are important pathogens to keep in mind in the services of Obstetrics and Gynecology. We carried out a descriptive and prospective study in order to investigate the presence of such organisms in 2763 endocervical samples of infertile patients. All clinical samples were analyzed by the MYCO Well D-GNE[®] of CPM Scientifica and a Multiplex-PCR method. The 69% of clinical samples were positive to urogenital mycoplasmas, of them 54% were positive to Up, 36.3% to Uu and 9.7% to Mh. Positive ureaplasma samples showed antimicrobial resistance to clarithromycin (16.6%) tetracycline (14.7%), rifaximin (11.2%), pefloxacin (0.9%) and minocycline (0.7%). *Mycoplasma* positive samples were resistant to azithromycin (68.1%), tetracycline (19.2%), minocycline (4.2%) and doxycycline (2.1%). Our results showed a high frequency of infection by *U. parvum* and *U. urealyticum* in infertile women. Furthermore it is being necessary to carry out studies of the ureaplasma serovar.

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Detection of *Ureaplasma parvum* and *Ureaplasma urealyticum* in HIV-1 infected women.

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The detection of *U. parvum* and *U. urealyticum* in endocervical specimens from HIV-1 infected women, and its relationship with other cervicovaginal infections and 3 age groups was investigated in patients. As a negative control, a group of HIV-1 negative women were studied. The 28.3% of the