Bulgarian Journal of Veterinary Medicine (2008), **11**, No 4, 219–234

PORCINE RESPIRATORY DISEASE COMPLEX (PRDC): A REVIEW. II. DIAGNOSTICS, TREATMENT AND PREVENTION

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Summary

Bochev, I., 2008. Porcine respiratory disease complex (PRDC): A review. II. Diagnostics, treatment and prevention. *Bulg. J. Vet. Med.*, **11**, No 4, 219–234.

The diagnostics of porcine respiratory disease complex (PRDC) requires a complex investigation with compulsory use of a serological assay (enzyme-linked immunosorbent assay, ELISA). The polymerase chain reaction (PCR) assay is also becoming more and more important. Prevention plays an essential role for eradication of this disease complex. The treatment with antibiotics is less important. The control measures should be complex and are mainly focused on restriction and hygiene. They include parent herds' closure, schedules for early weaning of piglets (segregated or medicated early weaning), the all-in/all-out management technology, periodical serological surveys etc. Vaccinations result in relatively weak effect against the main PRDC agents. The use of live vaccines in PRRSV interferes with the diagnostics and generates a risk of virulentization of the vaccinal strain whereas killed vaccines are not sufficiently immunogenic and therefore also impede diagnostics. The weak immunity in *Mycoplasma* infections is mainly due to the epithelial localization of pathogens, that makes them hardly accessible for host immune defense. The non-specific prevention is also prevailing for control of other bacterial agents of PRDC.

Key words: *Actinobacillus pleuropneumoniae* (APP), *M. hyopneumoniae*, pigs, porcine circovirus type 2 (PCV-2), porcine reproductive and respiratory syndrome virus (PRRSV), respiratory complex, segregated early weaning (SEW), vaccine

PRDC is a polyetiological disease in swine with a significant economical importance. In a previous publication (Bochev, 2007) we have reviewed in detail the etiology, epidemiology, clinical forms and pathoanatomical features of the disease complex. The present review will put an emphasis on PRDC diagnostics, treatment and prevention.

DIAGNOSTICS

Due to non-specific clinical and gross pathological signs, the precise diagnostics with consideration of the most important causative agents of the disease requires data from all possible tests. The complex diagnostics should begin with gathering of clinical and epidemiological information about the management system, age, origin of animals, parent health, vaccination history, morbidity and mortality rates, response to medications applied with prevention or treatment purposes (Honnold, 1999).

Diagnostic gross pathology

It is the leading among non-laboratory methods. The primary macroscopic findings of the gross pathology examination

and the slaughterhouse inspection are the most informative. The number of necropsied animals should be as higher as possible due to various findings (Thacker, 2002). For this purpose, some severely ill animals could be slaughtered and fresh organs (lungs and lymph nodes) – obtained. If more than 15% of lungs are affected, the chance of occurrence of enzootic pneumonia in the herd is considerable. The herds, free of *M. hyopneumoniae* exhibit rarely pneumonic alterations in more than 1% of pigs and even then, they are very insignificant (Motovski, 2003a; 2004a).

Clinical diagnostics

Because of the polyetiological nature of the disease, the clinical signs are variable, non-specific and could not be used independently for diagnostic purposes. Similarly to diagnostic pathology, examination of numerous animals is advised. The most apparent clinical sign in mycoplasmic infection is cough, the intensity of which is essential for diagnostics. It is evaluated by a score system as follows: score 0 – total lack of cough in fattening pigs during moving; score 1 – less than 10% of pigs exhibit sporadic cough; score 2 – cough is present in 10%–15% of fattening pigs and it persists during moving; score 3 – persisting cough in more than 50% of pigs (Yeske, 2003).

The accompanying reproductive disorders in the breeder herd are a sign of involvement of the porcine reproductive and respiratory syndrome virus (PRRSV). The infection with the swine influenza virus (SIV) could also result in high incidence of abortions.

Laboratory diagnostics

It provides the most complete information for both the number and species of microbial agents and the immune status of the herd in general. The specimens obtained from live animals are nasal and tracheal secretion, blood serum, colostrum. The serological tests are leading with regard to correct diagnosis. In mixed rearing systems, blood samples should be obtained from pigs at a different age despite their health status, with the number of samples from each age group depending on the supposed prevalence of the disease.

Detection of M. hyopneumoniae. The isolation of *M. hyopneumoniae* from organ samples is still considered as "gold standard" (Thacker, 2004). It however requires special nutrient media supplemented with antiserum against the common contaminant *M. hyorhinis,* that grows much faster than *M. hyopneumoniae* and overgrows it. In general, the isolation of *M. hyopneumoniae* is very difficult, slow and not always successful (Ross, 1999). That is why by now, slaughterhouse inspection and serological methods, mainly enzyme-linked immunosorbent analysis (ELISA), and an incresing number of genetic methods as polymerase chain reaction (PCR) are preferred for large-scale diagnostics. Blockade ELISA is more sensitive than indirect ELISA, but both tests are highly specific. The sensitivity of ELISA is not sufficient 3 to 6 weeks from the beginning of infection and is not efficient for diagnostics in vaccinated animals (Erlandson *et al.*, 2005). In herd diagnostics by means of blockade ELISA with monoclonal antibodies, the herd is considered affected if at least 10% of animals react positively and when single positive samples are detected, the herd is considered suspicious (Ala-Risku *et al.*, 2004). The immunofluorescence and immunoperoxidase tests provide a lot of false negative results. One-step PCR is most widely used as a routine diagnostic test, but it could not detect mycoplasmae in nasal

secretion, but only in tracheal or pulmonary specimens (Pijoan, 2002). At present, the qualitative nested PCR is the most sensitive. It could detect DNA from at least 4 *Mycoplasma* cells but is not suitable for broad use because of frequent contaminations (Pijoan, 2002). An advantage of PCR vs serological tests is that the former is not influenced by the presence of vaccinal antibodies.

Detection of bacterial pathogens. The diagnostics of *Pasteurella multocida* is relatively simple and is done by isolation on routine nutrient media – blood and McConkey's agar. The identification of organisms is performed mainly on the basis of their biochemical behaviour, and in case of *S. suis,* serotyping is necessitated. Of all bacterial pathogens, the detection of *Actinobacillus pleuropneumoniae* (APP) is the most difficult. The routine bacteriological examination of nasal and tonsil samples is not sensitive, as APP is often overgrown by banal microflora in samples and thus, the visual identification is very difficult. That is why, the use of immunomagnetic separation, nuclease analysis and PCR that are 1000 times more sensitive than separation, is recommended (Chiers, 2003).

Detection of PRRSV. The serological diagnostics of porcine reproductive respiratory syndrome (PRRS) includes the immunofluorescence test (IFT), immunoperoxidase monolayer assay (IPMA), ELISA, virus neutralization test (VNT). The serological analysis shows only the presence of infection but not systemic immune defense. It should be done prior to vaccination, as tests do not distinguish between infectious and vaccinal antibodies (Benfield *et al.*, 1999). An advantage of ELISA is the potential for rapid analysis of numerous samples as well as its ability to detect both American and European viral strains unlike the IFT or IPMA. The difficult isolation of PRRSV makes PCR more and more preferred because of its rapidity, high sensitivity and relative independence of the state of studied material. A positive result in PCR could be obtained up to 9 days earlier than in ELISA. With the introduction of automated PCR, the cost of extensive studies has been considerably reduced.

Detection of SIV. The isolation of influenza viruses is relatively easier compared to that of PRRSV and could be done by infection of 9–10-day old chick embryos with nasal secretion that should be clear and obtained during the febrile stage (Easterday & Van Reeth, 1999). The detection is simple by means of the haemagglutination test too. The determination of the type of viral haemagglutinin is performed by the haemagglutination inhibition test with reference sera and that of neuraminidase – by neuraminidase activity inhibition test. Some avian influenza viruses could also infect pigs without formation of detectable antibodies (Hinshaw *et al.*, 1981). Among more contemporary assays, ELISA and PCR, as well as the rapid immunoassay membrane test are widely used.

In diagnosing PRDC, some blood laboratory parameters, for example blood serum haptoglobin, could be used. This is an acute-phase protein and its concentration increases significantly in respiratory diseases, lameness, diarrhoea and cannibalism (Petersen & Nielsen, 2002).

Detection of porcine circovirus-2 (PCV-2). As an etiological agent of PRDC, PCV-2 provokes aggravation of respiratory signs and extensively slows growth (Neumann *et al.*, 2002). In laboratory diagnostics, the detection of specific microlesions and immunohistochemical methods is accepted as "gold standard".

By now, PCR has a primary importance in the detection of this virus.

TREATMENT

The treatment is practically possible only in cases of mycoplasmic and bacterial infections and is primarily targeted at mycoplasmae.

Chemotherapy of mycoplasmic infection

It is most successfully done by means of broad spectrum antibiotics from the tetracyclines, pleuromutilins and lincosamides families and their combinations in particular. In the USA, chlortetracycline or/and tiamulin, lincomycin, bacitracin premix are most commonly used. A plus of these antibiotics is their activity against the other bacterial agents of lung infection. Among the newer drugs, apart quinolones, the pleuromutilin valnemulin is reported to exhibit a very high activity against *M. hyopneumoniae* (Hannan *et al.*, 1997). *P. multocida* and *M. hyopneumoniae* are sensitive to doxycycline (Bousquet *et al.*, 1997) that, together with the activity of this antibiotic against *A. pleuropneumoniae* (Pijpers *et al.*, 1991), makes it appropriate for prevention of mixed infections. Tulathromycin is able to accumulate in lungs in concentrations 60 times higher than plasma ones. There it persists in bactericidal concentrations up to 5 days for *P. multocida* and up to 15 days for *M. hyopneumonae*. It is applied once, intramuscularly (Bosch, 2004). In experimental infection with *M. hyopneumonae*, APP serotype 2 and *P. multocida*, the best effect was achieved with the application of a medication premix containing valnemulin + chlortetracycline, followed by tiamulin + chlortetracycline, tilmicosin, chlortetracycline + procaine penicillin + sulfadimidine and finally, lincomycin + chlortetracycline (Stipkovits *et al.*, 2001). Due to the specific localization of mycoplasmae (adherence on respiratory epithelium), chemotherapeutics arrive more hardly at them and the successful therapy requires relatively high doses and a more prolonged treatment (at least 2–3 weeks), compared to bacterial infections (Bradford, 2002).

Chemotherapy of APP infection

APP is sensitive mainly to beta-lactams and co-trimoxazole (Taylor, 1999). It is also sensitive to tilmicosin (Paradis *et al.*, 2004), that although reducing considerably the bacterial numbers in tonsils, could not eliminate completely the carriership and the spreading (Fittipaldi *et al*., 2005). The antibiotic resistance, often observed with serotypes 1, 3, 5 and 7, is rarely encountered with serotype 2 (Taylor, 1999).

Chemotherapy of S. suis infection

In *in vitro* studies, *S. suis* exhibited the highest sensitivity to penicillin, amoxicillin, ceftiofur, florfenicol and gentamicin (Marie *et al.*, 2002). In mixed PRRSV and *S. suis* infection in weaned pigs, the lowest mortality rates occurred after injection with ceftiofur compared to oral administration of tiamulin, injection with procaine penicillin and individual vaccinations against both agents (Halbur *et al.*, 2000).

PREVENTION

The control of PRDC is difficult owing to its multifactorial etiology. Most pathogens could hardly be controlled and some of them (*P. multocida, S. suis*) are impossible to be eradicated. The prevention measures are general and specific ones. The determination of all microorganisms involved in the etiology of the disease in a

given farm is essential for setting up measures of control. There are numerous techniques for control and eradication of respiratory diseases: herd closure with vaccination or field virus infection for equalization of the immune status (elimination of subpopulations); diagnostical surveys with culling of infected animals; medicated and segregated early weaning; partial and complete depopulation (Dufresne, 2002). These techniques are frequently combined depending on the individual case.

The long-term strategy for PRDC control includes stabilization of the parent herd, strict adherence to the all-in all-out (AI-AO) procedure, segregated early weaning (SEW) and age-segregated housing (Halbur, 1997). The early weaning up to the age of 14–17 days is more and more applied as a universal tool for interruption of transmission of infections from the sow to suckling pigs. In SEW, the pigs are only weaned and moved to another premise or farm (Harris, 1988), whereas medicated early weaning (MEW) implies injection with antibiotics of preweaning pigs and of sows prior to and after farrowing (Alexander, 1982). For MEW performance, older sows from a closed parent herd are selected (after 3rd farrowing) and are moved into an isolated farrowing house. The farrowing is induced in order to ensure timely treatment of the offspring with antibiotics. The piglets are weaned at 5–7 days and only the biggest and healthy ones are kept. The rearing of early weaned pigs requires a lot of efforts for both housing and feeding. They are highly susceptible to infections because of the poor immune status and this necessitates the provision of ideal rearing conditions. A drawback of early weaning (under the age of 14 days) is the appearance of ovarian cysts in primiparous sows that results in nymphomania or anoestrus for several weeks and additionally increases the production costs (Britt, 1995, Mabry *et al.*, 1996). A MEW variant is modified medicated early weaning (MMEW), where unlike MEW, sows are not farrowed in an isolated house.

Measures against PRRSV

Most restrictive measures should be aimed against PRRSV, as its control is the most difficult. All programmes for disease eradication should begin with determination of the serological profile of the different structures of the farm. It is recommended to carry out an initial PCR screening of suckling piglets at the age of 7–18 days. They are selected among the offspring of younger sows because of the higher probability of virus transmission. If the PCR test is negative, then ELISA could be run to establish a contact with the virus and seroconversion (Gillepsie & Caroll, 2003). ELISA is effective if no vaccination has been performed as it could not distinguish between vaccinal and infectious antibodies. The serological monitoring of eradication programme in stock herds should be performed on a monthly basis. In the beginning, the number and distribution of samples should be such as to allow detection of 5% incidence of infection with 95% confidence. In the later stages, detection of 19% prevalence at 85% confidence could be admitted (Van Alstine *et al.*, 1993). The farms proved to be negative should be supplied with replacement pigs only from similar farms. All newly arrived replacement animals should be quarantined for at least 30 days. The isolation premise should be outside the farm and is visited by the end of the working day. By that time, the animals within are assayed serologically twice – at their arrival and 14 days later

for immune status determination with regard to PRRSV, *M. hyopneumoniae* and SIV. Infected farms need to stabilize the sow herd by means of natural or artificial immunization (Firkins, 1998). Prior to introduce seronegative replacement gilts in a positive herd, an acclimatization of newly arrived animals is advised. It is done by purposeful infection of new sows with PRRSV in isolation 90 days prior to their introduction in the main herd. This is achieved by isolation for 10–15 days with animals for culling or contact with manure from the farm (Morrow & Roberts, 2000). Then followed a period of development of the infection and recovery. Replacement sows should originate from a herd with stable parameters. The boars and the semen for artificial insemination should have the same origin (Torremorell & Baker, 1999). By means of acclimatization, young sows are infected and recover without shedding the virus, build immunity and thereafter that could be included in the main breeder herd (Morrow & Roberts, 2000). By the end of acclimatization period, replacement pigs could be vaccinated against the virus of the Aujeszky's disease (pseudorabies) and PRRSV. Acclimatization should be performed for own female replacement pigs as well (McCaw, 1995). In both the isolation and production premises, the all-in all-out principle should be observed, i.e. replacement animals should be introduced more rarely, but at larger batches. There is no need of acclimatization for introducing pigs, seropositive against the same strain than the breeder herd. McCaw (1995) proposes the McREBEL (Management Changes to Reduce Exposure to Bacteria to Eliminate Losses) system for PRRSV control. It is applicable in farms with separate premises for the different ages both at farrowing and in newly weaned piglets, using the all-in all-out system:

1. Cessation of cross-fostering of newborn piglets among recently farrowed sows for equalization of litters or preservation of sick and unthrifty piglets. The cross-fostering could be performed only within the first 24 hours of life.
2. The piglets and sows should not be moved outside the farrowing premise.
3. Cessation of using nurse sows for weak, PRRSV-infected and unthrifty piglets.
4. Minimization of piglets' handling with regard to antibiotic treatment or injections of iron preparations, as they often result in transmission of bacterial infections.
6. Immediate destruction of all severely ill pigs whose complete recovery is not possible.
7. Sick or undergrown piglets should not be retained with or mixed with younger piglets in a given premise.
8. Newly weaned piglets should strictly observe the all-in all-out technology. Two or three days should be allowed among batches for wet disinfection and drying.
9. The premises for newly weaned piglets could be loaded at a time with earlier weaning of some of oldest and biggest litters.

According to the author, the McREBEL system reduces considerably the economical losses, but not always succeeded to eliminate the virus from the farm.

The parent herd could be also stabilized by vaccination with a live attenuated vaccine. Live vaccines should not be used in pregnant sows because of the vertical transmission and in boars for less than 28 days of their utilization for insemination because of the shedding of vaccinal virus

(McCaw, 1995). The vaccination of the breeder herd could be combined with its closure. Herd closure could last for 5-8 months, during which the introduction of replacement pigs is discontinued, and only semen for artificial insemination is introduced. During that period, the subpopulations of pigs with different immune and infectious status and with decline in virus activity are eliminated. Closure could be prolonged with insemination and pregnancy of new sows outside the main herd and their returning back immediately prior to farrowing (Torremorell & Baker, 1999). The healing of stock animals should continue only after breeder herd stabilization.

Two main strategies are utilized for control and eradication of PRRSV-infection in weaned piglets: partial depopulation and vaccination (Benfield *et al.*, 1999). In continuous flow systems, a partial depopulation should be used whereas in the multi-site system – a total one (Baekbo, 2000). The vaccination protocols against PRRSV are yet disputable. The main disadvantage of all vaccine brands is the lack of crossed protection against the great variety of viral strains, which could occur simultaneously in the same farm (Baker, 2006). The vaccinal immunity is built up slowly, and it develops faster and stronger against strains, antigenically similar to the vaccinal one. The replacement sows should be vaccinated with a live vaccine twice at 1–2-month interval, and the revaccination should precede breeding by at least one month (Benfield *et al.,* 1999). Sows could be vaccinated 2 weeks after farrowing (McCaw, 1995). Vaccination of suckling pigs could be done once at 7 days of age with killed vaccine provided that there is a circulating virus in the farm and there are no colostrum antibodies, and twice (if there are interfering colostrum antibodies) at the age of 2–7 days and at weaning. The purpose is to start building immunity as early as possible due to its slow development. Live vaccines are more immunogenic than killed ones (Gillepsie & Caroll, 2003), but their use is not recommended in virus-free herds, as they cause reproductive disorders in sows and make the serological monitoring impossible. In killed vaccines, there is no risk of circulation of a vaccinal strain, but the built immunity is weak and inadequately protective (Pol & Steverink, 2000). They also impede the serological monitoring, and that is the reason for the development of a second generation of vaccines – subunit and vector DNA vaccines, but at present the former are not efficient (Pol & Steverink, 2000; Fernandez *et al.*, 2003).

Control of M. hyopneumoniae

Many of the general measures for PRRSV control could be also used for control of the mycoplasma infection. Attempts for its eradication are made long ago with a various success. Some of the first attempts were for isolated rearing of litters of older sows (after the third farrowing), presuming that older sows are free of infection and their offspring are free as well. (Goodwin & Whittlestone, 1967). Although many of these new herds appeared free of mycoplasmae (on the basis of slaughterhouse inspection and cultural studies), many became infected later again. By now, it is not known whether these early achievements were due to using litters of older sows or to isolated housing. PCR investigations of nasal swab samples showed considerable numbers of *Mycoplasma*-positive adult sows in normal herds (Calsamiglia *et al.*, 1999; Calsamiglia & Pijoan, 2000). Today, the at-

tempts for eradication of *M. hyopneumoniae* are mainly focused in two directions:

1. Segregated and medicated early weaning (SEW and MEW). These techniques could be only used to create new herds without being able to heal infected ones.

2. Partial depopulation of animals younger than 10 months combined with antibiotic medication of the others for 14 days. The empty premises should be cleaned and disinfected. The separated young pigs with a high genetic potential could be reared separately and after reaching the age of 10 months, are medicated and returned back to older pigs (Baekbo *et al.*, 1994). This technique is appropriate for smaller farms with a continuous flow technology, where the total depopulation is not beneficial. In 1996, in order to prevent the aerogenic reinfection in Switzerland, an obligatory programme for disease eradication has been implemented in many regions of the country by simultaneous partial depopulation and medication of farms and control of animal introduction in them. The results showed that up to the end of 2002, in regions where the eradication was successfully performed, new cases of infection have emerged in the years that followed, although with a low incidence (Stärk *et al.*, 2004). The authors believe that in the future, the number of *M. hyopneumoniae*-positive farms will decrease. A similar programme was executed in Finland in 1998–1999 as a pilot project with healing of 81% of farms (Heinonen, 2001).

Conforming to the models of PRDC development in the different production systems and the concept for 50% infection threshold, STOMP (Serologic Targeting of Medication Programs) has been developed for prevention of the bacterial manifestation of the disease (Greiman, 2000). STOMP aims to maintain the number of infected animals and the bacterial load in them by medication with antibiotics under the critical threshold until slaughtering. With regard to an economically effective medication and an as high as possible antibacterial effect of antibiotics, periodical serological surveys are performed in some animals to determine the rough number of infected pigs. Blood sample tests should be conducted at the ages of 6, 10, 14, 18 and 22 days. PCR test of a smaller sample of animals is also advised in order to establish the interaction between *M. hyopneumoniae* and PRRSV as well as to detect the time of the real spreading of microorganisms. Antibacterials should be administered just before reaching the critical 50% infection threshold in clinical outbreaks of infection. As a result, the period of treatment is maximally shortened and thus, medication costs are reduced. For a higher efficacy, pulsed medication is performed, that consists of antibiotic administration for 5–7 days at higher doses alternated with 1–2 week intervals of treatment. In the first PRDC pattern of early nursery infection, a twofold pulse medication is advised, the first pulse being at the age of 5–6 weeks and the second – by the age of 10–11 weeks. In the second disease pattern, with clinical manifestation by the age of 12–16 weeks, continuous medication is recommended between the end of growing and beginning of fattening or pulsed medication with first dose by the end of weaning and a second one by the age of 13–14 weeks. In the third, slowest disease pattern, continuous medication is advised during the first three weeks of the fattening. In systems with late manifestation of infection, metaphylaxis could be also used (Kolb, 2002). It consists in a short-time medication after natural infection in order

to interrupt the incubation process prior to the clinical manifestation of the disease. The pulsed medication, apart being less expensive, permits the contact with mycoplasmae and rise of an immune response during periods without treatment (Walter *et al.*, 2000, Kolb, 2002). Vaccination is widely used to control mycoplasmic infection, but as with PRRSV, there are some limitations. Only inactivated vaccines against *M. hyopneumoniae* are produced from whole cells – bacterins. These vaccines could not protect from infection and colonization with *Mycoplasma hyopneumoniae* and could not eliminate the carriership (Le Grand & Kobisch, 1996), although they reduce significantly pulmonary lesions – up to 93% (Groth & Rapp-Gabrielson, 2001). No correlation was found out between the presence of serum antibodies and protection from clinical illness (Thacker *et al.*, 2000a). After vaccination, serum antibodies usually decrease in the absence of infection and seronegativity could occur 4–6 weeks later (Thacker & Thanawongnuwech, 2002). Thacker & Thacker (2001) determined a various effect of maternal antibodies upon both natural and vaccinal immunity in their offspring. Antibodies from naturally infected sows protect the litter from disease, but interfere with the development of vaccinal immunity. Vaccinal antibodies of non-infected sows do not reduce the immunity of piglets, but a reduction was found to occur in infected and vaccinated sows (that is practically the general case). That is why, the regular vaccination of sows in mixed and one-site rearing systems is not recommended, but only allowed in the three-site system (Pijoan, 2002), where it could be combined with pulsed medication. A serological analysis is recommended to determine the most appropriate moment for vaccination of pigs, thus obtaining information for the immune status of sows too (Thacker & Thanawongnuwech, 2002). PRRS infection, occurring at the same time or shortly after vaccination against mycoplasmae, reduced considerably the efficacy of the vaccine. The same research has shown that the vaccination against *M. hyoneumonae* lessened the gravity of pneumonia, provoked by *M. hyopneumoniae* and PRRSV co-infection, whereas the vaccination with a live attenuated vaccine against PRRSV did not exhibit this effect. The vaccination with a live attenuated vaccine against PRRSV prior to vaccination against mycoplasmae resulted in reduced effect of the second vaccine in some instances and no such effect in others (Thacker *et al.*, 1999; Thacker *et al.*, 2000b). These results indicate that the time of vaccination against mycoplasmae should be carefully chosen with regard to the vaccination against PRRSV. The decision for vaccination and the time of its performance depend on numerous factors – presence of *M. hyopneumoniae* in the farm, constant level of respiratory diseases, presence of primary and secondary infections with PRRSV, the virus of the Aujeszky's disease, APP, serious microbism, need for constant medication via the feed, varying and small weight gain, mortality rate over 4% from weaning to slaughtering (Motovski, 2003a).

For overcoming of problems caused by maternal antibodies and the effect of PRRSV, DNA vaccines and vaccines with special adjuvants should be implemented to ensure an effective protection in field conditions even on the background of high level of colostrum antibodies that do not interact with DNA (Thacker & Thanawongnuwech, 2002).

Control of SIV

Apart the usual zooprophylactic measures, the contact with migrating birds and men, suspected to be infected with influenza, should be restricted. The antibiotic therapy has a positive effect on clinical manifestation (Thacker, 2000). Vaccination is widely used in western Europe as a means of prevention. Inactivated whole virus or subunit oil emulsion vaccines are used against H1N1 A/New Jersey/1/76 and H3N2 according to the strain detected in the farm, because of the existence of several different H3N2 strains. There are monovalent and bivalent vaccines. Combined vaccines provide a protection against the H1N1 strain as well. Autogenous vaccines are also produced, but their efficacy is low (Thacker, 2000). Prior to vaccination, immune profiling of the whole farm is advised by means of ELISA or the haemagglutination inhibition (HI) test. Blood samples from at least 10 replacement pigs, 10 first-parity sows, 10 second-parity sows, 30 multiparous sows should be obtained. Also, 10–20 samples from recently weaned pigs at the age of 3–4 weeks are obtained. The geometric mean S/P ratio for first-parity and second-parity sows and for replacement sows in relatively healthy herds should be about 1.5–1.6 i.e. very close to ratios in older sows that are usually about 1.6 or higher. In recently weaned pigs, titres should be by about 0.1 lower that those of sows. In the HI test, antibody titres over 1:10 against H1N1 are considered protective against disease and rather high for successful vaccination. For H3N2 the allowed titre of colostrum antibodies is up to 1:40. Due to the common genetic drift of H3N2, a more frequent serological survey is recommended (Erickson, 2001). Vaccination of sows is advised, with twofold injection of replacement sows prior to breeding and infection of pregnant sows 30 days prior to each farrowing. In twofold vaccination with a monovalent vaccine, maternal antibodies are protective for the litter up to the 12th weeks of life (Thacker, 2000). Vaccination of stock pigs is done less frequently, as the economical effect is less pronounced or absent. In this category of pigs, a single vaccination at the age of 12–16 weeks is performed. The early weaned pigs with the least level of immunity should be vaccinated at an earlier age. As with mycoplasmosis, DNA vaccines against SIV are also developed, but the outcomes are still not promising (Larsen & Olsen, 2002).

Control of PCV-2

There are several types of killed vaccines and recombinant vaccines for specific prevention against the porcine circovirus-2 (PCV-2): Circovac® (Mérial), Porcilis® PCV (Intervet) etc. Circovac®, that is allowed for use in Bulgaria, is applied in replacement pigs and sows twice at a 3–4-week interval. Porcilis® contains only a viral capsid and is applied twice in small recently weaned pigs. The clinical trial of the vaccine Ingelvac CircoFLEX® in the UK showed almost 4 times reduction of mortality rates in vaccinated animals and over 6 kg higher weight gain compared to non-vaccinated pigs (Von Richthofen *et al.*, 2007).

Control of secondary bacterial microflora

The already described general zoohygienic measures: strict adherence to technology and to the all-in all-out principle, maintenance of optimal microclimate in premises, quarantine and examination of new animals etc., should be applied to control secondary bacterial microflora too. The prevention of pasteurellosis is aimed to restrict the spread of infection.

There are polyvalent killed vaccines (bacterins) against *P. multocida* biotypes A and D, combined with anatoxins of the same types for control of atrophic rhinitis. The efficacy of these vaccines is inadequate in field conditions due to the short-time immunity, raised only to homologous strains (Pijoan, 1999). Subunit vaccines are developed from outer membrane proteins, but commercial preparations are still not available (Ruffolo *et al.*, 1998).

In APP control, it is essential to prohibit introduction of infection in disease-free farms. This could be done by introducing animals from herds established to be healthy or from infected farms, but with hysterectomy procured animals (Taylor, 1999). The control of infected farms is done by treatment with antibiotics, vaccination and general means of prophylaxis, including periodical disinfection. A regular serological monitoring is also performed. The eradication of APP is very difficult. It could be achieved through depopulation and replacement with pigs established to be healthy or gradual replacement of infected animals with pigs, hysterectomy produced and artificially reared in isolation. Both alternatives are expensive (Taylor, 1999). In farms with fewer seropositivity rates (up to 30%), a more conservative eradication protocol could be followed – periodical survey with elimination of seropositive animals, accompanied by antibiotic therapy (Nicolet, 1970; Nielsen *et al.*, 1976). In this procedure, pregnant sows are sampled shortly before farrowing, and piglets are weaned at the age of 2 weeks, strictly isolated from potentially infected animals. These, that remain seronegative until the age of 12 weeks, are used as replacement pigs. Positive sows are methodically eliminated until the complete healing of the herd. This programme lasts for 6–12 months.

Numerous vaccines against APP are manufactured, divided in two groups: killed and subunit. The killed vaccines are serotype-specific, and subunit ones – polyvalent. They are applied twice after decline of colostrum antibodies. Vaccines do not always remove carriership, but improve the general healthy status of animals and thus, have a positive economical impact (Taylor, 1999).

In Bulgaria, Yordanov (2001) has developed a programme for prevention and therapy of APP infection, that includes both general and specific measures: control of the introduction of pigs, application of the all-in all-out technology, hygienic measures, restriction of stress factors, bacteriological and serological investigations, prophylactic antibiotic use, vaccination of weaned pigs and of sows in breeder farms with subunit vaccines, isolation and treatment of diseased pigs with antibiotics, slaughtering of seriously ill pigs and sanitization of carcass and slaughtering wastes. The used subunit vaccine provided a better protection compared to bacterins (Motovski, 2004b). Through the application of this programme, healing of several industrial pig farms has been achieved in a period from 5 months to 1 year (Yordanov, 2001).

Motovski (2003a; 2003b) has pointed out the principal means that should be realized for PRDC control in our conditions, as follows:

1. Determination of the etiology of PRDC for every farm.

2. Improvement of the general hygienic conditions in the farms and especially those of weaned pigs.

3. Twofold vaccination against the Aujeszky's disease in infected farms at the ages of 10 and 14 weeks.

4. Maintenance of a high immunity against PRRSV in the main herd by closure of farm, constant or periodical vaccinations (in cases of acute manifestation of disease – reproductive and respiratory disorders), vaccination of replacement animals prior to their introduction in the farm.

5. On the background of the developed stable immunity against PRRSV in the herd and colostral immunity in pigs, respectively, to test vaccination of pigs against PRRS, APP, enzootic pneumonia, individually or in combinations, and then, to apply the most appropriate alternative (Motovski, 2003b).

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Paper received 12.09.2007; accepted for publication 16.06.2008

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