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HEALTH & CONSUMERS DIRECTORATE-GENERAL
Unit 04 - Veterinary Control Programmes

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Programmes for the eradication, control and monitoring of certain animal diseases and zoonoses

Eradication programme of Aujeszky's Disease

Approved* for 2010 by Commission Decision 2009/883/EC

Bulgaria

* in accordance with Council Decision 2009/470/EC

REPUBLIC OF BULGARIA
MINISTRY OF AGRICULTURE AND FOOD

PROGRAM

for control and eradication of Aujeszky disease in pigs in the Republic of Bulgaria in 2010

Contents:

1. Identification of the programme.

Members state:	**Republic of Bulgaria**
Disease:	**Aujeszky disease in pigs (AD)**
Year of implementation:	**2010**
Reference of this document:	National Veterinary Service – NVS
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2. Historical data on the epidemiological evolution of the disease:

Aujeszky disease (AD) in Bulgaria was first found in 1947 in both bovines (Nachev & Petrichev) and pigs (Semerjiev). Genev и Stoyanov (1957) were the first in the world to prove the involvement of the Aujeszky disease virus (ADV) in the aetiology of respiratory diseases of pigs. In the first 10 years after the first occurrence the disease developed a high prevalence among the pig population of the country (Toneva - 1958). The consolidation of the pig industry and the establishment of large cooperative pig holdings was done by collecting the animals from small pig holdings whereupon animals infected with the AD virus were kept together with uninfected animals. The large industrial pig holdings were created by importing animals from the British company PIC Camborough into nucleus farms and reproducing them in breeding and stock pig farms. Due to the fact that nearby pig farms infected with Aujeszky disease were authorized to continue their operations the disease was introduced into such farms as well.

In all three stages of its spreading Aujeszky disease caused huge economic losses to the pig industry in Bulgaria - abortions in pigs, respiratory diseases in pigs and costs for prophylactic vaccination and medication therapy.

What contributed to the spreading of Aujeszky disease in intensive pig farming the pyramidal structure of such intensive systems whereby young breeding animals for stock farms were bought from breeding farms and in turn the animals for the breeding farms were bought from nucleus farms. In that situation the infection of the nucleus farm would lead to infection of all pig holdings at the lower levels of the pyramid – breeding and stock pig farms.

A large number of pig holdings were affected by AD, including small family type pig farms and backyard herds intended for personal consumption, as a result of the purchase of pigs from large pig holdings.

The cases of backyard fattening of one pig do not represent an epizootic or economic issue.

Often the source of infection of a newly infected farm remained unidentified, which comes to suggest that airborne virus transmission in winter also had a role to play in the spreading of the virus.

What also contributed to the wide spreading of AD was than many farms used vaccination as replacement for the general prophylactic measures (i.e. the latter were ignored).

In order to limit the losses vaccination was introduced, as government policy, using originally inactivated (ethanolsaponin) and subsequently - live attenuated vaccine. For many years each

pig was vaccinated twice a year and each fattening pig – once. Thanks to this, clinical manifestations of the disease were rarely to be observed but the virus persisted in pig holdings.

What largely contributed to the persistence of the virus in the affected industrial pig farms was that such farms performed both breeding operations and fattening of the newly born piglets, which became the major reservoir for the virus. This was reinforced by the fact that dozens thousands of animals were reared in industrial pig farms.

The results of the virological investigations (virus isolation in cell cultures) performed at the National Diagnostic Research Veterinary Institute (NDRVI) in the recent 10 years show that disease breakdowns occurred in all those years, 62% of the tested animals were positive for antibodies with positive tests results of the newly born piglets, which suggests that the virus persisted among the sows. This assumption is confirmed by the fact that the disease agent was isolated in the tested foetuses. All pigs showing clinical signs of the disease tested during the period were positive, which is indicative of the Aujeszky disease prevalence. The virus was also isolated from clinically healthy sows, which comes to suggest persistence of the disease agent. Before that period AD used to be confirmed by virological testing that covered sheep and bovines. This shows that in Bulgaria too the pigs infected by the AD virus constitute a permanent threat for the other animal species.

Though few, the mentioned virological investigations are indicative, as they were not performed in the context of a monitoring programme for the disease but in response to a disease suspicion .

Serological screening was not performed either – instead, generally imported animals were tested, and the results of such tests are by far not indicative. The conducted testing of 20 blood samples from each of 14 large pig holdings shows that more than 42% of the tested pig holdings and more than 34% of the tested animals were positive for antibodies, which might be due to the administered vaccinations. Almost all pig holdings and most of the animals in them were serologically positive, which is evidence of administered vaccinations. Due to the small number of tested animals it cannot be stated with certainty that the remaining pig holdings are free of the disease. It is likely however that, as a result of the many years of vaccination and the dramatic decrease in the number of animals in the holdings, some of them have become free of the virulent virus. A large proportion of the industrial pig holdings were subjected to depopulation and repopulation of breeding stock from the other member countries and no vaccination against Aujeszky disease was performed in the repopulated holdings .

The intensity of the epizootic process of AD varies across pig holdings and age groups, in each of them however it takes the course of latent infections without the characteristic nervous and influenza-like signs. The major form of manifestation of the disease is respiratory diseases and mass reproductive disorders caused by intrauterine infection of foetuses.

The Bulgarian isolates of the AD virus belong to genotype I according to the classification of Herrmann et al (1984). They differ in terms of virulence, organotropism and restriction profile of the genome.

Three attenuated vaccines against AD in pigs were administered successively in Bulgaria (Tatarov, 1968): MK-25 (1970 – 1980), MK-35 (gE+) (1980 – 1990) and MK-35 (gE-) (after 1990). All three vaccines are thymidine kinase positive (TK+), the last one MK-35 (gE-) being identical with the Bartha K-61strain. They differ in virulence for cell cultures and rabbits (Manov and Motovski, 1994). On the 85th day of the pregnancy transplacental infection was induced by administering higher MK-35 (gE) doses. Recombination between MK-35 (gE+) and MK-35 (gE-) was achieved in cell cultures and rabbits (Manov and Motovski, 1994). Derivates of MK-35 (gE+) were isolated from pigs (Gielkens & Berns, 1982). Another specific feature of the Bulgarian vaccines is that the vaccine virus is dissolved in salt solution instead in

oil adjuvant and swine are vaccinated twice a year and pigs – once a year. Thus the resulting immunity is not sufficiently sustained, which is favourable for development of sub-clinical and inapparent infections.

After Bulgaria became a full EU member the requirements for securing guarantees for free intercommunity trade requires implementation of a Programme for Monitoring and Eradication of Aujeszky disease throughout the country. The purpose of this Programme is to conduct a mass scale screening of the population of domestic and East-Balkan pigs and to implement the relevant measures for control and eradication of the disease.

For the purposes of this Programme the pig holdings are divided into the following categories:

a/ Industrial holdings – large pig holdings operating a closed production cycle and implementing biosecurity measures ;

b/ Family holdings type "A" – small commercial farms operating an open production cycle and implementing biosecurity measures;

c/ Family holdings type "B" - small commercial farms not implementing biosecurity measures;

d/ backyard pig holdings – pigs reared under extensive farming systems and exclusively intended for own consumption;

e/ East-Balkan pig herds reared under pasture conditions on the territory of three country regions;

This categorization of pig holdings in Bulgaria is based on a risk analysis.

The present programme will cover all pig holdings implementing biosecurity measures as these have the potential to trade with other EU member countries. Another focus of the programme will be the testing of pigs reared in small commercial farms not implementing biosecurity measures. In the framework of the programme special attention will be paid to the East-Balkan pig herds reared on pastures as these present a risk of indirect contacts with wild pigs.

3. Description of the submitted programme:

In order to ensure additional guarantees in respect of AD in pig trade this Programme for Control of AD in 2010 will be implemented in all pig holdings throughout the territory of the Republic of Bulgaria. The key components of this programme were developed on the basis of a comprehensive scientific analysis, taking account of the specific features of the pig industry in Bulgaria, namely:

a) reliable data are available, which show that the wild AD virus is in circulation among the pig population of Bulgaria which affects all categories of pig holdings, including the East-Balkan herds;

b) in 12 municipalities in three country regions East-Balkan pigs are reared under pasture regime, in herds and therefore direct or indirect contact with the wild pig population cannon be excluded;

c) the results of the genotyping of virus isolates prove that they belong to genotype I according to the classification of Herrmann et al (1984).

The key elements of this programme are :

1. Monitoring of pigs for AD, including sampling and testing of a sufficient number of blood samples from each pig holding to determine the status of the holding in respect of Aujeszky disease.

2. Vaccination against AD with highly immunogenic, high-titre adjuvant marker (gE negative and TK – negative) vaccines, ensuring sustained herd immunity to stop the spreading of the wild virus among the animals in the affected holding.

3. Testing of organ samples /lungs and tonsils of infected pigs and swine, lings and liver of foetuses/ from clinically healthy pigs and aborted foetuses by PCR or virus isolation .

3. Fast and effective implementation of the measures for control and eradication of the disease form infected pig holdings.

4. Scientific analysis of the epidemiological data, of currently implemented and prospective future measures for control and eradication of AD in Bulgaria.

4. Measures of the submitted programme

4.1. Summary of measures under the programme

Duration of the programme: 01.01.2010. – 31.12.2013

First year:		Last year:	
■	**Control**	■	**Eradication**
■	**Testing**	■	**Testing**
□	**Slaughtering of positive animals**	□	**Slaughtering of positive animals**
□	**Killing and Rendering of positive animals**	□	**Killing and rendering of positive animals**
■	**Vaccination**	□	**Slaughtering**
□	**Treatment**	□	**Disposal of products**
□	**Disposal of products**	■	**Monitoring**
■	**Monitoring**	□	**Other**

4.2. Designation of the central authority charged with supervising and coordinating the departments responsible for implementing the programme:

This programme will be implemented by:

4.2.1. The National Veterinary Service (NVS), Ministry of Agriculture and Food

- The Animal Health Directorate at NVS Headquarters shall be responsible for:
  - Developing and detailing the Programme for Control and Eradication of AD in 2010 - 2013 г;
  - Coordinating the actions of all bodies involved in the implementation of the programme ;
  - Collecting information and preparing reports on the results of the Programme implementation;
  - Reporting the Programme implementation to the Commission.
- The heads of the Animal Health departments at the Regional Veterinary Services (RVS) shall be responsible for:

- Supervising the implementation of the programme at regional level. They shall supervise the activities of all bodies involved at regional level and shall report on the programme implementation to the Headquarters of NVS;

- The official veterinarians in charge of the supervision of meat processing establishments are responsible for:
  - The ante-mortem and post-mortem inspection in compliance with the rules laid down in Council Regulation (EC) 854/2004, Art 5 and Annex I. In addition they perform documentary and identity checks;
  - The health marking of meat;
  - The sampling of pigs for AD diagnosis in compliance with the 2010 sampling scheme, ensuring traceability of samples back to the holding of origin ;
  - Notifying NVS of any suspected case of AD /manifestation of clinical signs in pigs during the ante-mortem inspection / and sending the samples to the National Reference Laboratory for AD in Sofia;
  - In cases of AD – for declaring the meat from the pigs unfit for human consumption in line with the provisions of Council Regulation (EC) 854/2004, Annex I, Section II, Chapter V, Point 1(e) and seizing the meat in an appropriate manner;
  - Inspecting the sanitation and disinfection of transport means used for transportation of pigs.
- The official veterinarians in charge of animal health at municipal level shall be responsible for:
  - Implementing and supervising the programme implementation within the relevant municipality ;
  - Drafting the reporting documents and reports on the implementation of the programme ;
  - Supervising the complete coverage of all pig holdings for the purposes of the programme by the registered veterinary practitioners in the process of sampling and vaccination against AD;
  - In the event of any suspicion of AD -- taking and sending samples for virological testing ;
  - Performing documentary checks and identity checks in the context of pig movements within the territory of Bulgaria,
  - Ensuring traceability of samples back to the holding of origin ,
  - Monitoring the improvements and implementation of biosecurity measures in holdings, including the sanitation and disinfection procedures, based on the biosecurity principles of the holding;
  - Implementing and supervising the implementation of the rules for registration of animal holdings, pigs identification and movement control, including closing holdings and confiscating pigs in case of violation or non-conformity with the rules set forth in Council Regulation (EC) 882/2004,

4.2.2 The National Reference Laboratory (NRL) for AD in Sofia shall be responsible for:

- Testing the received blood serum samples by gE ELISA and organ samples by PCR and virus isolation;
- Recording the test results in the IT system of NVS;
- Reporting the test results to the Headquarters of NVS;
- Communicating the test results to the relevant Regional Veterinary Service and registered veterinary practitioner sending the samples for testing;
- Performing investigations by methods described in the Manual for Diagnostic Tests and Vaccines of OIE;
- Sending samples for genotyping of virus isolates to the EU Reference Laboratory;
- Participating in ring tests organized by the EU Reference Laboratory for AD.

4.2.3 The registered veterinary practitioners shall be responsible for

- Implementing this programme in the pig holdings they are in charge of ;
- The daily monitoring of the health status of pigs;
- Taking and sending samples for laboratory analysis;
- implementing emergency vaccination at the direction of the Regional Veterinary Service;
- performing clinical examinations and issuing veterinary movement certificates for pigs depending on the results of the laboratory analysis for Aujeszky disease.

4.2.4 The Associations of pig breeders shall have the following responsibilities:

- To actively support the implementation of this programme by:
- Ensuring awareness of all members of the association for the purposes of this programme;
- Providing the necessary number of samples for the implementation of this programme;
- Ensuring vaccination against AD when directed by the official veterinarians of the Regional Veterinary Service.

4.3 Description and delimitation of the geographical and administrative areas in which the programme is to be implemented

The programme will be enforced on the whole territory of Bulgaria, involving all of its 28 districts.

4.4. Measures implemented under the programme.

4.4.1. Measures and terms of legalisation as regards the notification of the disease :

Aujeszky disease is a notifiable disease according to Art. 50 of the Law on Veterinary Activities.

4.4.2. Target animals and pig population covered by this programme

This programme covers all types of pig holdings – industrial; type A farms implementing biosecurity measures and type B farms not implementing biosecurity measures, East-Balkan pig herds and wild boars.

Blood samples for serological testing are taken in the field by the registered veterinary practitioners and the official veterinarians controlling the meat processing establishments during slaughter of pigs.

The number of blood samples is determined on the basis of the table attached hereto, as follows:

- from boars – 100%;
- from sows – 5% prevalence and 95% confidence level;
- from the other pig categories – full coverage - 10% disease prevalence and 95% confidence level;
- from pigs that have died on farms with diagnosed seropositive reagents – organ samples /lungs and tonsils from animals that showed clinical signs of the disease or from animals that have died or foetuses in case of abortion / for virological testing and AD virus isolation ;
- from pigs originating in backyard farms – samples are taken in case of manifested clinical signs or epizootic data (communication with AD-affected pig holdings) for Aujeszky disease.
- from wild boars – the samples taken from wild boars during the implementation of CSF programme will be tested also for Aujeszky disease.

4.4.3 Measures and terms of legislation as regards the registration of holdings and identification of animals:

In Art.51 of the Law on Veterinary Activities it is stipulated that animals are subject to identification and the holdings are subject to registration. NVS is the official competent authority responsible for the identification of the animals. A computerized information system supports the recording and processing of the data concerning the identified animals and their movements as well as the registered holdings and electronic herd register. The NVS information system is use for the necessary traceability of the sampling and testing process and the recording of the active clinical surveillance of the domestic pig population.

Ordinance No 61 transposes Council Directive 92/102/EEC into Bulgarian legislation. Art 3 sets out the details concerning the identification of Animals and the registration of holdings: The registration of the holdings (including the pig-keeping ones) is carried out by the authorities of the National Veterinary Service (NVS) or by veterinary specialists - private practitioners duly authorized by the NVS.

All animal holdings, where large ruminants, small ruminants or pigs are kept are subject to mandatory registering in:
1.The registry on animal movement control that is kept by the owner;
2.The NVS information system.

The code (serial) registration number of the holding is provided once and stays the same no matter of the species and types of animals kept in that holding. All data shall be kept at

least three years after the cessation of the activity of certain animal holding or after sale, slaughter, death etc. of the last animal kept in that holding.

The new categorisation of pig holdings in the context of the eradication of Aujeszky disease described under 2) above has no effect on the obligation of pig farmers or keepers as regards holding/herd registration, the identification of animals and their movements.
Ordinance No 61 also lays down the terms and procedures to be complied with concerning the identification of the animals.

Pigs are identified by individual ear-tag (bearing either unique serial number or, in case of fattening pigs intended for direct slaughter, the registry number of the pig holding of fattening). Any such ear-tags must be and are delivered and distributed by the NVS. Displacing or reuse of ear-tags placed on an animal is not permitted. The identification of the animals has to be carried within 20 days after birth or when living the holding at least. Only in case of pigs for fattening in industrial farms the identification can be carried out at the latest before they leave for slaughter with green ear-tags indicating the number of the holding.

According to the Law on Veterinary Activities (LVA), owners of farm animals are obliged to:

- ensure availability of their animals to be identified /ear-tagged/, for vaccinations and for diagnostic test purposes;
- ensure access to animal holding and the animals therein for the state veterinarian inspecting them;
- comply with and meet the requirements related moving and transportation of animals.

There is a ban imposed on any movement of unidentified animals to markets, slaughterhouses, fairs, exhibitions, competitions or other holdings or settlements.

4.4.4 Measures and terms of legislation as regards the different qualifications of animals and herds:

The pigs herds and pigs covered by this program are shown by category in the tables below:

Industrial holdings

Region	Number of holdings	Number of pigs
Blagoevgrad	-	-
Burgas	3	13 791
Varna	4	38 600
Veliko Turnovo	3	14 500
Vidin	1	4 146
Vratsa	1	40
Gabrovo	1	2 135
Dobrich	1	11 634
Kurdzhali	-	-
Kystendil	-	-

Lovech	2	10 266
Montana	1	4 637
Pazardzhik	3	20 300
Pernik	-	-
Pleven	2	1 540
Plovdiv	2	6 900
Razgrad	1	34 000
Ruse	7	89 813
Silistra	4	25 500
Sliven	2	11 951
Smolyan	-	-
Sofia-city	-	-
Sofia-region	1	2 850
Stara Zagora	5	41 181
Targovishte	3	15 316
Haskovo	1	1 200
Shumen	9	63 627
Yambol	5	11 333
Total for the country:	**61**	**425 260**

Family type category A farms

Region	Number of holdings	Number of pigs
Blagoevgrad	1	32
Burgas	-	-
Varna	2	52
Veliko Turnovo	11	5 600
Vidin	2	230
Vratsa	-	-
Gabrovo	5	775
Dobrich	3	255
Kurdzhali	1	10
Kystendil	2	64
Lovech	5	1 712
Montana	5	590
Pazardzhik	6	4 900
Pernik	-	-
Pleven	1	360
Plovdiv	3	1 650
Razgrad	2	1 190
Ruse	3	1 335
Silistra	4	629
Sliven	4	2 439
Smolyan	-	-
Sofia-city	2	256

Sofia-region	-	-
Stara Zagora	5	901
Targovishte	1	439
Haskovo	-	-
Shumen	4	658
Yambol	7	1 254
Total for the country:	**79**	**24 331**

Family type category B farms

Region	Number of holdings	Number of pigs
Blagoevgrad	52	2 958
Burgas	86	2 351
Varna	15	629
Veliko Turnovo	35	2 600
Vidin	67	404
Vratsa	10	604
Gabrovo	47	420
Dobrich	98	1 449
Kurdzhali	12	199
Kystendil	26	201
Lovech	29	1 736
Montana	41	441
Pazardzhik	2	155
Pernik	-	-
Pleven	122	2 284
Plovdiv	50	2 083
Razgrad	15	2 150
Ruse	26	1 110
Silistra	13	528
Sliven	20	1 059
Smolyan	18	382
Sofia-city	44	732
Sofia-region	96	3 010
Stara Zagora	180	2 305
Targovishte	68	5 443
Haskovo	30	1 200
Shumen	11	1 569
Yambol	128	695
Total for the country:	**1 341**	**38 697**

Backyard pig holdings

Region	Number of backyard holdings	Number of pigs
Blagoevgrad	3 485	5 384
Burgas	1 582	3 135
Varna	2 014	6 787
Veliko Turnovo	1 479	2 366
Vidin	2 352	5 552
Vratsa	2 987	5 632
Gabrovo	412	898
Dobrich	2 177	3 925
Kurdzhali	3	5
Kystendil	3 793	5 387
Lovech	1 192	1 599
Montana	1 338	1 933
Pazardzhik	2 120	3 248
Pernik	1 239	4 725
Pleven	4 155	8 577
Plovdiv	1 546	2 665
Razgrad	76	257
Ruse	126	215
Silistra	897	2 355
Sliven	8 583	14 432
Smolyan	75	168
Sofia-city	81	167
Sofia-region	2 350	4 050
Stara Zagora	3 525	6 080
Targovishte	628	1 884
Haskovo	750	3 000
Shumen	604	1 201
Yambol	1 218	1 908
Total for the country:	**50 787**	**97 535**

Eat-Balkan pigs

Region	Number of herds	Number of pigs
Burgas	25	1 029
Varna	59	5 061
Shumen	23	2 494
Total:	**107**	**8 584**

Wild boar population

	Region	No of wild pigs in state hunting areas	No of wild pigs in areas provided for hunting to the National Union of Hunters and Anglers	Total
Hunting Areas according to the Regional Forestry Directorates	Berkovitsa	550	2 719	3 269
	Burgas	2 126	1 957	4 083
	Blagoevgrad	1 545	2 853	4 398
	Varna	1 157	2 108	3 265
	Veliko Tarnovo	1 499	3 543	5 042
	Kardzhali	643	3 992	4 635
	Kyustendil	759	2 667	3 426
	Lovech	1 448	3 406	4 854
	Pazardzhik	1 511	1 170	2 681
	Plovdiv	1 191	1 957	3 148
	Ruse	918	1 710	2 628
	Sliven	1 200	3 126	4 326
	Smolyan	557	2 758	3 315
	Sofia	1 468	5 497	6 965
	Stara Zagora	659	1 787	2 446
	Shumen	1 110	1 909	3 019
	total	**18 341**	**43 159**	**61 500**
National parks	Rila National Park (NP)	498		
	Pirin National Park	404		
	Central Balkans NP	940		
	UOGS	115		
	Voden Hunting area	291		
	MNO	25		
	Iskar Hunting area	190		
	total	**2 463**		
Total Year 2009		**63 963**		

4.4.5. Control procedures and in particular rules on the movement of animals liable to be affected or contaminated by a given disease and the regular inspection of the holdings or areas concerned:

The Bulgarian Traceability database will be used for control of pig's movement in the context of the Ajuesky programme . The respective obligations of pig holders as regards the registration of holdings, the identification of the pigs and their movement control are described above.

Rules on the movement of animals

According to the national veterinary legislation of the Republic of Bulgaria movement of live animals is subject to a prior clinical examination and temperature measuring. Animals are identified by an ear tag, each consignment being accompanied by a movement certificate based on a model approved by the Director General. In connection with the implementation of the approved Programme for Control and Eradication of Aujeszky disease each pig consignment is additionally accompanied by a completed check list of the conducted clinical survey pf the pig holding of origin. The transport means used for transportation of pigs shall be licensed, mechanically cleaned and disinfected.

4.4.6. Measures and terms of legislation as regards the control (clinical surveillance, testing, vaccination,) of the disease:

All blood samples are taken using individual needles in single use vacuum containers.

All samples are accompanied by accompanying letters and a list of individual ear tags corresponding to the information on the vacuum containers.

Samples are transported in cool boxes in impervious containers.

Samples are not deep frozen but are stored at refrigeration temperatures.

They are delivered to the laboratory as quickly as possible.

Tissue and organ samples are placed in hermetically sealed and duly labelled polythene bag. The packaged samples are then placed in big resistant containers wrapped with absorbing material sufficient to protect the container against damage and absorbing any possible liquids. Where possible the containers are delivered directly to the laboratory by the competent persons to ensure fast and reliable transportation.

The following information shall appear on the external side of the packaging: the address of the receiving laboratory and the following warning on a visible place: "Pathologic material of animal origin. Perishable. Fragile. To be opened solely at the laboratory for AD in pigs."

Samples are sent to the Viral Diseases of Pigs Laboratory at the Virological Section of NDRVI in Sofia, 15 Pencho Slaveykov Str.

Blood samples are tested by the complementary gE ELISA method, which allows distinguishing vaccinated from infected animals.

Detection of the virus by isolation in cell cultures and of nucleic acid by polymerase chain reaction.

The virological testing of received organ samples is to be carried out by the methods: PCR and virus isolation.

Marker vaccination

In case of positive serological results with gE ELISA, the official veterinarian immediately directs vaccination with marker vaccine of the pig population on the farm based on the following scheme:

Sows and boars – every 4 months;

Gilts – first vaccination – at 10 weeks of age, second vaccination – at 14 weeks of age and third vaccination – at 6 months of age;

Fattening pigs – first vaccination – at 10 weeks of age and second vaccinations– at 14 weeks of age.

Every 6 months new samples are taken from the farms for serological testing by gE ELISA.

Cessation of marker vaccination is ordered after two consecutive negative results of tests, performed 6 months from each other, for presence of antibodies by gE ELISA.

4.4.7. Vaccines and vaccinations schemes used

Emergency scheme for vaccination of a holding with positive samples tested by gE ELISA in a laboratory:

- sows and boars – every 4 months;

- gilts – first vaccination – at the age of 10 weeks, second vaccination – at the age of 14 weeks and third vaccination – at the age of 6 months;

- fattening pigs – first vaccination – at the age of 10 weeks and second vaccination - at the age of 14 weeks.

The costs of vaccination with marker vaccine are covered by the owners of the holding.

Every 6 months new samples are taken from the holding for serological testing by gE ELISA.

Cessation of marker vaccination is ordered after two consecutive negative results of tests, performed 6 months from each other, for presence of antibodies by gE ELISA.

Based on obtained positive results of the preformed serological screening by gE – ELISA and based on a risk analysis the Director of the Regional Veterinary Service orders emergency vaccination with marker vaccine of the pig population in the affected holding. The pig holding is considered free after two consecutive serologically negative results of tests, performed 6 months from each other by gE ELISA.

Vaccination data (date and vaccine type) vaccinated animals are entered into the IT system of NVS.

Only marker vaccines licensed for use in the Republic of Bulgaria are used.

4.4.8 Information and assessment on bio-security measures management and infrastructure)in place in the holdings involved:

During the active surveillance for the emergence and spread of the disease, Aujeszky disease, through clinical examinations of holdings a special check-list is drafted for checking the biosecurity measures introduced in the holdings. On the basis of biosecurity measures introduced for the purposes of this Schedule, the holdings are divided into: Industrial farms / farms with biosecurity measures and closed cycle of rearing pigs, Family farms, type "A" - these are the farms open cycle of rearing pigs with biosecurity measures, Family farms, type "B" - farms with uninitiated or partially implemented biosecurity measures, "back yards" - private farms, which rear to 5 pigs for personal consumption, "East Balkan pigs" - population, which is rear only in the territory of 12 municipalities in 3 regions of the country, under certain conditions that ensure the biosecurity of pigs. In the gaps in the biosecurity measures introduced in carrying out clinical examination, the official veterinarian give a prescription for a period to correct deficiencies of the owner of the holding.

4.4.9 Measures and terms of legislation in case of a positive result

In case of positive serological results, to determine the wild AD virus circulation, sampling is performed for virological testing. Positive serological results obtained from gE – ELISA testing are completely sufficient evidence of wild virus circulation and no virological testing is necessary.

In case that the presence of the AD virus is confirmed in a holding the official veterinarian in charge undertakes the following steps:

- review of the register and the identification marks of the pigs on the farm, placing the farm under official ban and ordering:

- a ban on the trade in live animals with the exception of animals intended for immediate slaughter;
- vaccination of the whole population with maker vaccine based on a vaccination scheme;
- movements of pigs for immediate slaughter shall be accompanies by a veterinary certificate containing the following text: "the animals originate in a holding with diagnosed Aujeszky disease, the animals were vaccinated and the withdrawal period of the vaccine has elapsed";
- where new pigs are introduced in the holding these shall be subjected to a 30-day quarantine in premises specially designated for the purpose and shall be attended by separate staff. During the quarantine period the pigs shall be vaccinated;
- introduction of new pigs shall be prohibited unless there are appropriate conditions for quarantine;
- taking of organ samples /lungs and tonsils/ from pigs that have died or pigs showing clinical signs of the disease;
- notifying the other owners of pig holdings in the 3 and 10-kilometre zone around the infected holding of the diagnosed Aujeszky disease;
- implementing the biosecurity measures in all pig holdings in the 3 and 10-kilometre zone around the holding and in the affected holding;
- a holding shall be considered free after two consecutive negative serological results from tests performed 6 months from each other;

4.4.10. Procedure for compensating owners of slaughtered and killed animals

No compensation.

4.4.11. Control of program implementation and reporting

The control of this program is implemented at local level by the official veterinarian of the respective municipality, at regional level – by the Director of the Regional Veterinary Service and the head of the Animal Health Department and at national level – by the Animal Health Directorate at the Headquarters of NVS.

Programme implementation is reported to the European Commission in Brussels.

The current programme will be reported in accordance with Commission Decision 2008/940/EC. Two reports are performed: intermediate in July and final for the whole year.

5. Benefits of the Programme

The implementation of this programme will make it possible to provide sufficient guarantees in respect of the health status of pigs in Bulgaria as regards Aujeszky disease, as well as unobstructed trade in live animals in case of amendment of Commission Decision 2008/855/EC and reduced risk of AD spreading within the EU.

6. Data on the epidemiological evolution
6.1. Evolution of the disease
6.1.1. Data on evolution of the disease
6.1.1.1. Data on herds

Year : 2004 **Situation on date:** 31/12/2004
Disease: Aujeszky **Animal species:** domestic and East-Balkan pigs

Type of pig holding	Total number of herds	Total number of herds under the programme	Number of herds checked	Number of positive herds in 2004	Number of new positive herds	Number of herds depopulated	% positive herds depopulated	Indicators		
								% herd coverage	% positive herds Period herd prevalence	% new positive herds Herd incidence
1	2	3	4	5	6	7	8=(7/5) x100	9=(4/3) x100	10=(5/4) x100	11=(6/4) x100
Industrial holdings	76		5	4						
Small farms	410									
Backyard farms in places with 500 or more pigs	5467									
Backyard farms in places with less than 500 pigs	127900		1	0						
East-Balkan pigs	380									
Total:	**134 233**		**6**	**4**						

Year : 2005 **Situation on date:** 31/12/2005
Disease: Aujeszky **Animal species:** domestic and East-Balkan pigs

Type of pig holding	Total number of herds	Total number of herds under the programme	Number of herds checked	Number of positive herds in 2005	Number of new positive herds	Number of herds depopulated	% positive herds depopulated	Indicators		
								% herd coverage	% positive herds Period herd prevalence	% new positive herds Herd incidence
1	2	3	4	5	6	7	8=(7/5) x100	9=(4/3) x100	10=(5/4) x100	11=(6/4) x100
Industrial holdings	72		5	2						
Small farms	298									
Backyard farms in places with 500 or more pigs	4570									
Backyard farms in places with less than 500 pigs	110600									
East-Balkan pigs	330									
Total:	**115 870**		5	2						

Year : 2006 **Situation on date:** 31/12/2006
Disease: Aujeszky **Animal species:** domestic and East-Balkan pigs

Type of pig holding	Total number of herds	Total number of herds under the programme	Number of herds checked	Number of positive herds in 2006	Number of new positive herds	Number of herds depopulated	% positive herds depopulated	Indicators		
								% herd coverage	% positive herds Period herd prevalence	% new positive herds Herd incidence
1	2	3	4	5	6	7	8=(7/5) x100	9=(4/3) x100	10=(5/4) x100	11=(6/4) x100
Industrial holdings	83		9	2						
Small farms	373									
Backyard farms in places with 500 or more pigs	4981									
Backyard farms in places with less than 500 pigs	105279									
East-Balkan pigs	313									
Total:	**111 029**		**9**	**2**						

Year : 2007 **Situation on date:** 31/12/2007
Disease: Aujeszky **Animal species:** domestic and East-Balkan pigs

Type of pig holding	Total number of herds	Total number of herds under the programme	Number of herds checked	Number of positive herds in 2007	Number of new positive herds	Number of herds depopulated	% positive herds depopulated	Indicators		
								% herd coverage	% positive herds Period herd prevalence	% new positive herds Herd incidence
1	2	3	4	5	6	7	8=(7/5) x100	9=(4/3) x100	10=(5/4) x100	11=(6/4) x100
Industrial holdings	83		5	4						
Small farms	373		3	2						
Backyard farms in places with 500 or more pigs	4981									
Backyard farms in places with less than 500 pigs	105279									
East-Balkan pigs	313									
Total:	**111 029**		**8**	**6**						

Year : 2008 **Situation on date:** 31/12/2008
Disease: Aujeszky **Animal species:** domestic and East-Balkan pigs

Type of pig holding	Total number of herds	Total number of herds under the programme	Number of herds checked	Number of positive herds in 2008	Number of new positive herds	Number of herds depopulated	% positive herds depopulated	Indicators		
								% herd coverage	% positive herds Period herd prevalence	% new positive herds Herd incidence
1	2	3	4	5	6	7	8=(7/5)x100	9=(4/3)x100	10=(5/4)x100	11=(6/4)100
Industrial holdings	76		2	0						
Family type farms category A	74									
Family type farms category B	2546									
Backyard farms	72 402		1	0						
East-Balkan pigs	152									
Total:	**75 250**		**3**	**0**						

Year : 2009 **Situation on date:** 20/04/2009
Disease: Aujeszky **Animal species:** domestic and East-Balkan pigs

Type of pig holding Region Bulgaria	Total number of herds	Total number of herds under the programme	Number of herds checked	Number of positive herds in 2009	Number of new positive herds	Number of herds depopulated	% positive herds depopulated	Indicators		
								% herd coverage	% positive herds Period herd prevalence	% new positive herds Herd incidence
1	2	3	4	5	6	7	8=(7/5)x100	9=(4/3)x100	10=(5/4)x100	11=(6/4)100
Industrial farms	61		1	0						
Family type farms category A	79									
Family type farms category B	1 341									
Backyard farms	50 787									
East-Balkan pigs	107									
Total:	**52 375**		**1**	**0**						

6.1.1.2. Data on animals
Year: 2004 **Animal species - pigs**
Disease – Aujeszky

Region	Total number of animals (c)	Number of animals (d) to be tested under the programme	Number of animals (G) tested	Number of animals tested individually (c)	Number of positive animals	Slaughtering		Indicators	
						Number of animals with positive result slaughtered or culled	Total number of animals slaughtered (')	% coverage at animal level	% positive animals Animal prevalence
1	2	3	4	5	6	7	8	9 = (4/3)x100	10=(6/4)x100
BULGARIA	1 279 084								

Year: 2005 **Animal species - pigs**
Disease – Aujeszky

Region	Total number of animals (c)	Number of animals (d) to be tested under the programme	Number of animals (G) tested	Number of animals tested individually (c)	Number of positive animals	Slaughtering		Indicators	
						Number of animals with positive result slaughtered or culled	Total number of animals slaughtered (')	% coverage at animal level	% positive animals Animal prevalence
1	2	3	4	5	6	7	8	9 = (4/3)x100	10=(6/4)x100
BULGARIA	1 080 519								

Year: 2006 **Animal species - pigs**
Disease – Aujeszky

Region	Total number of animals (c)	Number of animals (d) to be tested under the programme	Number of animals (G) tested	Number of animals tested individually (c)	Number of positive animals	Slaughtering		Indicators	
						Number of animals with positive result slaughtered or culled	Total number of animals slaughtered (')	% coverage at animal level	% positive animals Animal prevalence
1	2	3	4	5	6	7	8	9 = (4/3)x100	10=(6/4)x100
BULGARIA	1 014 933								

Year: 2007 **Animal species - pigs**
Disease – Aujeszky

Region	Total number of animals (c)	Number of animals (d) to be tested under the programme	Number of animals (G) tested	Number of animals tested individually (c)	Number of positive animals	Slaughtering		Indicators	
						Number of animals with positive result slaughtered or culled	Total number of animals slaughtered (')	% coverage at animal level	% positive animals Animal prevalence
1	2	3	4	5	6	7	8	9 = (4/3)x100	10=(6/4)x100
BULGARIA	951 216								

Year: 2008 **Animal species - pigs**
Disease – Aujeszky

Region	Total number of animals (c)	Number of animals (d) to be tested under the programme	Number of animals (G) tested	Number of animals tested individually (c)	Number of positive animals	Slaughtering		Indicators	
						Number of animals with positive result slaughtered or culled	Total number of animals slaughtered (')	% coverage at animal level	% positive animals Animal prevalence
1	2	3	4	5	6	7	8	9 = (4/3)x100	10=(6/4)x100
BULGARIA	681 246								

Year: 2009 up to 20.04.2009 **Animal species - pigs**
Disease – Aujeszky

Region	Total number of animals (c)	Number of animals (d) to be tested under the programme	Number of animals (G) tested	Number of animals tested individually (c)	Number of positive animals	Slaughtering		Indicators	
						Number of animals with positive result slaughtered or culled	Total number of animals slaughtered (')	% coverage at animal level	% positive animals Animal prevalence
1	2	3	4	5	6	7	8	9 = (4/3)x100	10=(6/4)x100
BULGARIA	594 407								

6.2. Stratified data on surveillance and laboratory tests

6.2.1. Stratified data on surveillance and laboratory tests

Year: 2004 Animal species: domestic pigs

Disease : Aujeszky

Description of the used serological tests : gE ELISA

Description of the used virological tests: RT PCR, virus isolation in cell cultures

Region	Serological tests		Virological tests		Other tests	
	No of samples tested	No of positive samples	No of samples tested	No of positive samples	No of samples tested	No of positive samples
Republic of Bulgaria	0	0	31	22	0	0

Year: 2005 Animal species: domestic pigs

Disease : Aujeszky

Description of the used serological tests : gE ELISA

Description of the used virological tests: RT PCR, virus isolation in cell cultures

Region	Serological tests		Virological tests		Other tests	
	No of samples tested	No of positive samples	No of samples tested	No of positive samples	No of samples tested	No of positive samples
Republic of Bulgaria	0	0	61	10	0	0

Year: 2006 Animal species: domestic pigs
Disease : Aujeszky
Description of the used serological tests : gE ELISA
Description of the used virological tests: RT PCR, virus isolation in cell cultures

Region	Serological tests		Virological tests		Other tests	
	No of samples tested	No of positive samples	No of samples tested	No of positive samples	No of samples tested	No of positive samples
Republic of Bulgaria	0	0	92	20	0	0

Year: 2007 Animal species: domestic pigs
Disease : Aujeszky
Description of the used serological tests : gE ELISA
Description of the used virological tests: RT PCR, virus isolation in cell cultures

Region	Serological tests		Virological tests		Other tests	
	No of samples tested	No of positive samples	No of samples tested	No of positive samples	No of samples tested	No of positive samples
Republic of Bulgaria	0	0	58	49	0	0

Year: 2008 Animal species: domestic pigs
Disease : Aujeszky
Description of the used serological tests : gE ELISA
Description of the used virological tests: RT PCR, virus isolation in cell cultures

Region	Serological tests		Virological tests		Other tests	
	No of samples tested	No of positive samples	No of samples tested	No of positive samples	No of samples tested	No of positive samples
Republic of Bulgaria	0	0	24	0	0	0

Year: 2009 up to 20.04.2009 Animal species: domestic pigs
Disease : Aujeszky
Description of the used serological tests : gE ELISA
Description of the used virological tests: RT PCR, virus isolation in cell cultures

Region	Serological tests		Virological tests		Other tests	
	No of samples tested	No of positive samples	No of samples tested	No of positive samples	No of samples tested	No of positive samples
Republic of Bulgaria	0	0	22	0	0	0

6.3. Data on infection (one table per year and per disease / species)

Year: 2004 **Animal species: domestic and East-Balkan pigs**
Disease: Aujeszky

Bulgaria	Number of herds infected	Number of animals infected
Industrial farms	4	
Family type farms		
Backyard farms		
East-Balkan pigs		
Total:	4	

Year: 2005 **Animal species: domestic and East-Balkan pigs**
Disease: Aujeszky

Bulgaria	Number of herds infected	Number of animals infected
Industrial farms	2	
Family type farms		
Backyard farms		
East-Balkan pigs		
Total:	2	

Year: 2006 **Animal species: domestic and East-Balkan pigs**
Disease: Aujeszky

Bulgaria	Number of herds infected	Number of animals infected
Industrial farms	2	
Family type farms		
Backyard farms		

East-Balkan pigs		
Total:	2	

Year: 2007 **Animal species: domestic and East-Balkan pigs**
Disease: Aujeszky

Bulgaria	Number of herds infected	Number of animals infected
Industrial farms	4	
Family type farms	2	
Backyard farms		
East-Balkan pigs		
Total:	6	

Year: 2008 **Animal species: domestic and East-Balkan pigs**
Disease: Aujeszky

Bulgaria	Number of herds infected	Number of animals infected
Industrial farms		
Family type farms category A		
Family type farms category B		
Backyard farms		
East-Balkan pigs		
Total:	0	

Year: 2009 Situation on date – 20.04.2009 Animal species: domestic and East-Balkan pigs
Disease: Aujeszky

Bulgaria	Number of herds infected	Number of animals infected
Industrial farms		
Family type farms category A		
Family type farms category B		
Backyard farms		
East-Balkan pigs		
Total:	**0**	

6.4 N.A

6.5. Data on vaccination and treatment programmes

6.5.1 Prophylactic vaccination against Aujeszky disease

The table below shows the number of herds and pigs vaccinated in Bulgaria in the period 2004 - 2008 against Aujeszky disease

Year	Number of herds vaccinated	Number of pigs vaccinated
2004	38	291 631
2005	38	294 593
2006	41	309 406
2007	37	214 386
2008	31	132 700
2009 as of 20.04.2009	21	38 422

7. Targets

7.1. Targets related to testing

7.1.1. Targets on diagnostic tests

7.1.1.1 Number and specification of tests

The epidemiological aspects of AD are taken into account in order to define the objects of testing – herds and animals. From epidemiological perspective there are five categories of pigs in Bulgaria as defined above.

The table below presents the targets on testing of samples taken from all five categories of pigs under this program for surveillance and eradication of Aujeszky disease.

Targets on testing under this programme

Region	Type of test	Target population	Type of sample	Objective	Number of planned tests
Bulgaria	gE- ELISA	All categories	blood	monitoring	30 794
	Virus isolation	All categories	lungs and tonsils of diseased swine or pigs, lungs and liver of foetuses	confirmation of Aujeszky	100
	RT PCR polymerase chain reaction	All categories	lungs and tonsils of diseased swine or pigs, lungs and liver of foetuses	confirmation of Aujeszky	1 000

7.1.2 Targets on testing herds and animals

7.1.2.1. Targets on the testing of herds

Disease: Aujeszky **Animal species: domestic and East-Balkan pigs**

Type of pig holdings	Total number of herds	Total number of herds under the programme	Number of herds expected to be checked	Number of expected positive herds	Number of expected new positive herds	Number of herds expected to be depopulated	% positive herds expected to be depopulated	Target indicators		
								Expected % herd coverage	% positive herds Expected period herd prevalence	% new positive herds Expected herd incidence
1	2	3	4	5	6	7	8=(7/5)*100	9=(4/3)*100	10=(5/4)*100	11=(6/4)*100
Industrial	61	61	61	0	0	0	0	100,00	0	0
Family farms type A	79	79	79	0	0	0	0	100,00	0	0
Family farms type B	1 341	1 341	1 341	0	0	0	0	100,00	0	0
Backyards	50 787	50 787	50 787	0	0	0	0	100,00	0	0
East-Balkan pigs	107	107	107	0	0	0	0	100,00	0	0
Total	**52 375**	**52 375**	**52 375**	**0**	**0**	**0**	**0**	**100,00**	**0**	**0**

7.1.2.2. Targets on the testing of animals: 2010

Disease: Aujeszky **Animal species: domestic pigs, East-Balkan pigs and Wild boar**

Type of pig holdings	Total number of animals (c)	Number of animals (d) under the programme	Number of animals (d) expected to be tested	Number of animals to be tested individually ('-')	Number of expected positive animals	Slaughtering		Target indicators	
						Number of animals with positive result expected to be slaughtered or culled	Total number of animals expected to be slaughtered (f)	Number of animals with positive result expected to be slaughtered or culled	Total number of animals expected to be slaughtered (f)
1	2	3	4	5	6	7	8	9=(4/3)x100	10=(6/4)x100
Industrial	425 260	425 260	3 596	3 596	0	0	400 000	0,85	0
Family farms type A	24 331	24 331	4 582	4 582	0	0	20 000	18,83	0
Family farms type B	38 697	38 697	13 410	13 410	0	0	35 000	34,65	0
Backyards	97 535	97 535	0	0	0	0	97 000	0	0
East-Balkan pigs	8 584	8 584	6 206	6 206	0	0	8 000	72,30	0
Wild boar	63 963	3 000	3 000	3 000	0	0	0	0	0
Total	**658 370**	**597 407**	**30 794**	**30 794**	**0**	**0**	**560 000**	**4,68**	**0**

7.2 Targets on testing herds and animals

Disease: Aujeszky **Animal species: domestic and East-Balkan pigs**

Region: Republic of Bulgaria

Type of holdings	Total number of herds and animals under the programme		Targets on the status of herds and animals under the programme											
			expected unknown		Expected not free or not officially free from disease				Expected not free or not officially free from disease status suspended		Expected free from disease		Expected official free from diseas	
					Last check positive		Last check negative							
	Herds	Animals	Herds	Animals	Herds	Animals	Herds	Animals	Herds	Animals	Herds	Animals	Herds	Anima
Industrial	61	425 260	61	425 260	0	0	0	0	0	0	0	0	0	
Family farms type A	79	24 331	79	24 331	0	0	0	0	0	0	0	0	0	
Family farms type B	1 341	38 697	1 341	38 697	0	0	0	0	0	0	0	0	0	
Backyards	50 787	97 535	50 787	97 535	0	0	0	0	0	0	0	0	0	
East-Balkan pigs	107	8 584	107	8 584	0	0	0	0	0	0	0	0	0	
Total	**52 375**	**594 407**	**52 375**	**594 407**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	

7.3 Targets on vaccination or treatment

7.3.1. Targets on vaccination

Disease: Aujeszky **Animal species: domestic pigs**

Type of holdings	Total number of herds under the vaccination programme	Total number of animals under the vaccination programme	Targets of vaccination programme			
			number of herds under the vaccination programme	number of herds expected to be vaccinated under the vaccination programme	number of animals expected to be vaccinated under the vaccination programme	Expected vaccine doses
Industrial						
Family farms type A						
Total						

Remark: the vaccination will be performed as additional measure after the serological sampling of animals and determination of the possitive herds in different regions and type of pig holdings.

8. Detailed analysis of the costs of the programme:

Costs related to:	Specification	Number of units for 2010	Unitary cost in euro for 2010	Total amount in euro for 2010	Community funding requested (yes/no)
1. Testing					
1.1. Cost of the analysis	gE ELISA test antibodies	30 794	2.00	61 588,00	Yes
	Virus isolation	100	10.00	1 000.00	Yes
	RT PCR	1 000	15.00	15 000.00	Yes
1.2. Cost of sampling	Number of samples	30 794	1.00	30 794.00	Yes
1.3. Other costs packaging and transportation of samples to the laboratory				5 000.00	Yes
2. Vaccination or treatment					
2.1. Purchase of vaccine/treatment	-				
2.2. Distribution costs					
2.3. Administering costs					
2.4. Control costs					
***3.* Slaughter and destruction**					
3.1. Compensation of animals					
3.2. Transport costs					
3.3. Destruction costs					
3.4. Loss in case of slaughtering					
3.5. Costs from treatment of products (milk, eggs, hatching eggs, etc)					
4. Cleaning and disinfection					
5. Salaries (staff contracted for the programme only)					
6. Other costs				5 000.00	Yes
Total:				**118 382.00**	Yes

Explanation of the method of estimation of the number of samples under this programme

Method of estimation of the number of blood samples from pigs for AD testing

Number of pigs per unit or holding	AD - prevalence		
	20%	10%	5%
	Number of pigs to be tested (n)		
10	8	10	10
20	10	16	19
30	11	19	26
40	12	21	31
50	12	22	35
60	12	23	38
70	13	24	40
80	13	24	42
90	13	25	43
100	13	25	45
120	13	26	47
140	13	26	48
160	13	27	49
180	13	27	50
200	13	27	51
250	14	27	53
300	14	28	54
350	14	28	54
400	14	28	55
450	14	28	55
500	14	28	56
600	14	28	56
700	14	28	57
800	14	28	57
900	14	28	57
1.000	14	29	57
1.200	14	29	57
1.400	14	29	58
1.600	14	29	58
1.800	14	29	58
2.000	14	29	58
3.000	14	29	58
4.000	14	29	58
5.000	14	29	59
6.000	14	29	59
7.000	14	29	59
8.000	14	29	59
9.000	14	29	59
10.000	14	29	59
> 10.000	14	29	59

The table allows to estimate the individual number of pigs to be sampled for AD testing with 95% confidence level, whereby at least one infected animal can be found where the disease prevalence is 5%, 10% or 20%.