DETECTION OF INFECTIOUS BRONCHITIS VIRUS

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Introduction

Infectious bronchitis virus (IBV) is a major cause of economic losses in poultry and can be involved in respiratory disease, nephritis, and both poor egg production and quality. However, these signs are not specific to IBV. Therefore, diagnostic tools are needed to identify IBV infections in relation to a clinical problem in the field. This may also include typing of the isolate involved in order to enable the choice of a vaccination programme with the best chance of achieving sufficient protection against an IBV infection in the next flock.

In general, acute IBV infections can be diagnosed by detection of IBV virus (antigen) itself or the specific antibody response. The most common assays for routine use of virus detection are virus isolation (VI), immunofluorescence assay (IFA), immunoperoxidase assay (IPA), polymerase chain reaction (PCR), and for antibody detection the haemagglutination inhibition (HI) test, agar gel precipitation test (AGPT), and enzyme linked immunosorbent assay (ELISA). The virus neutralisation test (VNT) is rarely used for routine diagnosis because it is relatively expensive and laborious.

Choosing between tests and subsequent interpretation of the results can be very difficult and confusing and is quite often thwarted by the poor documentation of the performance of tests after IBV infections in the field. Depending on the demands and local circumstances, a best choice or best combination of techniques for that situation can be made. This paper attempts to present the current possibilities for detecting IBV infections, including discussion about the possible advantages and disadvantages of the available tests.

Detection of IB virus

Factors influencing the success of IBV detection

The level of success in detection of IBV after a disease outbreak is influenced by many factors of which the time between onset of infection and sampling, the level of immunity in the chicken at the moment of infection, and the number, choice and quality of sampled organs are the most important.

Time elapsed between onset of infection and sampling

The upper respiratory tract is the primary site of IBV replication, leading to viraemia and dissemination of the virus to other tissues (McMartin, 1993; Dhinakar Raj & Jones, 1997). All IBV strains can be isolated from the respiratory tract, with the highest concentration of IBV in the trachea during the first 3 to 5 days after infection. After this period, the virus titre drops rapidly in the second week post infection to below the detection level.

When chickens are sampled in the chronic stage of an IBV infection, one is more likely to isolate IBV from the intestinal tract (caecal tonsils or cloaca swabs) than from the trachea (Cook, 1968, 1984; Alexander & Gough, 1977; Alexander et al., 1978; El-Houadfi et al., 1986; De Wit et al., 1998).

A complicating factor from a diagnostic point of view is the question whether and if, in which organ(s), a persistent carrier state for vaccines and/or field viruses can develop. The two candidate sites mentioned for persistence are caecal tonsils and kidney. Although further studies are needed to know the nature of long-term infections and re-excretion, the phenomenon complicates the interpretation of IBV detection: The virus that is detected from an IBV suspected flock having been vaccinated or infected before, does not necessarily infer a recent infection. Therefore, even when IBV is detected in an IBV suspected flock, it is important to exclude other possible (infectious and non-infectious) causes of the disease.

Level of immunity in chicken at the moment of infection

The level of acquired immunity (by vaccination of previous infection) at the moment of infection has a major influence on the time and amount of IBV that can be detected (Figure 1 and 2). Experimental IBV infections in vaccinated and unvaccinated birds show that homologous challenge virus is detected during a much shorter period and at much lower (factor 10^2 - 10^4) levels than in unvaccinated chickens. The period during which IBV can be isolated from the trachea after a homologous infection of vaccinated birds can be limited to a few days instead of a few weeks after infection of unvaccinated birds.

These data show, that for attempting to detect IBV field virus from vaccinates, it is important to sample at an early stage of the infection and to use a very sensitive test.

Number, choice and quality of sampled organs

In the acute phase of an IBV infection of unprotected chickens, many birds will obtain high amounts of IBV in the trachea, so only a few birds have to be sampled. However, in case of chronic infections or infections in vaccinated birds, like layers and breeders, only low amounts of virus may be present in only a low percentage of the birds. Detection requires the sampling of respiratory tract, kidney, caecal tonsils and cloaca of many more birds.

To minimise the risk of inactivation of IBV in a carcass or sample, because of its limited thermostability, chilling of the material to 4°C should be done as soon as possible. If virus isolation (VI) is to be attempted within a day no other storage precaution is necessary. For longer storage the samples should be frozen at below -20°C as soon as possible. If freezing is not possible the tissues should be placed in special media and glycerol (Cumming, 1969). Under these conditions IBV will remain viable for many days, even if refrigeration is not available (McMartin, 1993).

Virus isolation (multiplication and / or detection of infectious IBV)

Virus isolation (VI) can be laborious, time-consuming and costly. Increasingly, the classical way of isolation, that is giving a number of embryo passages until dwarfing, curling or embryo mortality occur, is more often replaced

Figure 1: Average virus excretion by M41 infected unvaccinated broilers (Avian Pathology, 27, 464-471)
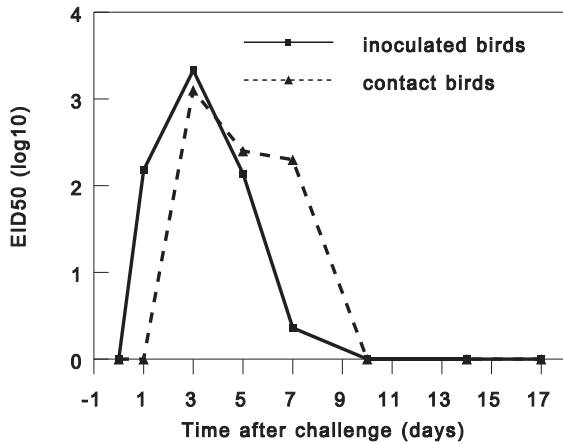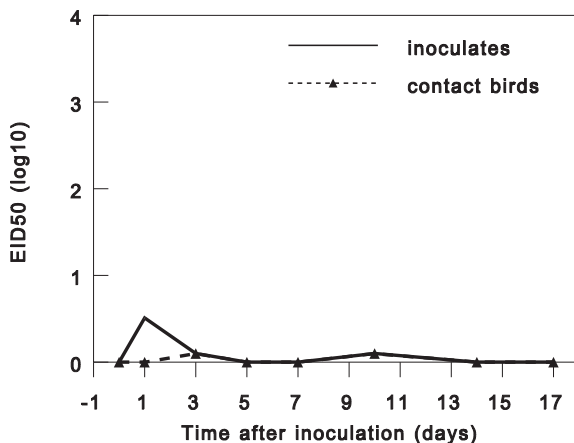

Figure 2: Average virus excretion by M41 infected H 120 vaccinated broilers (Avian Pathology, 27, 464-471)

by a combination of a shortened isolation procedure for multiplication of the virus and a second technique, such as IFA, antigen ELISA or PCR for antigen detection. By using the combination of isolation and other techniques, the procedure can be shortened with maintenance of sensitivity.

Field strains of IBV can be isolated using embryonated (SPF) eggs or trachea organ cultures (TOCs). In none of these systems, IBV causes specific lesions (McMartin, 1993). Therefore, the presence of IBV antigen has to be confirmed by an IBV antigen detection method.

Detection of IBV antigen

The techniques that are suitable for detection of IBV-specific antigen use IBV-specific antibodies. These antibodies are either in the form of antisera or monoclonal antibodies (Mabs). Antisera are from an animal that was infected with IBV or injected with certain parts of the virus. Consequently, antisera may contain antibodies against different parts of the virus. Standardisation and technical performance of antisera is hampered by the in vivo biological variation of the infected animal and virus. Since a Mab only reacts with one or a small number of epitope(s) of the IBV antigen, it provides a well-defined,

reproducible and specific product. A disadvantage of using a Mab to detect IBV antigen, or more accurately, a certain epitope, is that a mutation of only one nucleotide, resulting in a different amino acid in the epitope with which the Mab reacts, can prevent binding of the Mab to that epitope. Depending on the Mab used, this would suggest either that the strain is not IBV or is not of that serotype. This problem can be reduced by choosing Mabs against highly conserved or essential areas of the virus, or by using a mixture of Mabs. The risk, that one missing or changed epitope will cause a false negative result in an antiserum-based test is much smaller, when polyclonal antisera are directed against more epitopes.

The available tests for detecting IBV antigen will now be considered in turn.

Agar-gel precipitation test (AGPT)

The group-specific AGPT can be used for the detection of antigen. The test is very cheap, fast and requires few laboratory facilities. For best results, several antisera or an antiserum at different dilutions should be used, to prevent false negative results caused by imbalance of the antigen: antiserum ratio (Lohr, 1981). Although the test has an image of poor sensitivity, the published data using the test directly on organs suggest that it is not lower than that of recent techniques when used directly on organs. Another application of the AGPT is for confirmation of the presence of IBV in inoculated eggs (allantoic fluid or CAM). In this situation, the sensitivity will be increased due to the replication of the virus.

Immunofluorescent assay (IFA)

The IFA is a relatively cheap and fast technique for detecting IBV antigen in chickens, eggs, TOCs and cell culture. The IFA is group-specific when using polyclonal anti-IBV serum or group-specific Mabs. When using type-specific Mabs, the IFA can be a type-specific test (De Wit et al, 1995).

The interpretation of the fluorescent reactions may be complicated by non-(IBV)-specific reactions. The specificity of IFA can be improved by using Mabs instead of hyperimmune anti-IBV serum (Yagyu & Ohta, 1990; De Wit et al., 1995). Protection of the epithelium by reducing storage time (at low temperatures) between sampling and fixation will also improve the specificity of the IFA.

Because, unlike with VI, the antigen is not replicated before detection, the amount of antigen must be large enough to be detected by staining. Therefore, depending on the test material, the sensitivity can vary from equal (first week of infection in unprotected birds) to or much less than VI (chronic) infection in vaccinated birds.

The IFA can also be used for detection of IBV antigen in embryonated eggs, cell cultures, and TOCs.

Immunoperoxidase assay (IPA)

The IPA technique is, like the IFA, a staining technique with a comparable sensitivity to that of IFA. After fixation and embedding of sections of organs or tissues, an anti-IBV specific peroxidase-labelled conjugate binds with and stains the antigen in the sample, after addition of the substrate and chromogen. Advantages of IPA compared to IFA are, that IPA allows evaluation of antigen-

bearing cells as well as general tissue morphology. Also, evaluation of the slides can be done in day-light using a normal microscope, and storage of the slides is easy because of the stability of the staining, in contrast with IFA, where the staining fades when not stored dark and frozen. Disadvantages of IPA compared to IFA are that the technique is more laborious, takes a few days and is sensitive to non-specific background staining by endogenous peroxidase that is naturally present in the sample. This endogenous peroxidase has to be removed during the process of performing the IPA.

Antigen ELISA

Because of the high amounts of virus that are needed for detection by antigen ELISA, the sensitivity for detecting IBV antigen directly in chicken organs is low. Elisa is more suited as confirmation test for detecting IBV antigen in allantoic fluid of inoculated eggs, especially when a large number of samples has to be tested.

Detection of the IBV genome

Techniques that detect all or part of the IBV genome may be used for IBV detection. Although one- and two-step procedures are reported, the detection of genomic RNA is usually a three step procedure. The first step is replication of the virus from the (field) sample using embryonated eggs or TOCs. Subsequently, the genomic RNA is translated by reverse transcriptase (RT) into copy-DNA (cDNA) and multiplied many times by polymerase chain reaction (PCR). The last step is classifying the strain into a genotype using a third technique (see later).

Reverse transcriptase polymerase chain reaction (RT-PCR)

A technique increasingly used is the reverse transcriptase polymerase chain reaction (RT-PCR). The sensitivity of the RT-PCR is usually low when performed directly on organs. Therefore, IBV is often first multiplied in embryonated eggs or TOCs before the RT-PCR is performed. The RT-PCR product is to be identified as originating from IBV by another technique such as sequencing, restriction enzyme fragment length polymorphism (RFLP), or hybridisation. Further, use of appropriate controls to check the performance of the test and possible cross-contaminations are required.

Strain classification

Typing of IBV strains is useful for implementation of control measures, for research purposes and for understanding the epidemiology and evolution of IBVs. Classification of strains is hampered by the lack of standardisation of tests used world wide, use of different names for the same type of virus, the number of different test systems available and, maybe most important, the nature of this virus. A typing system suggests that a strain clearly belongs to only one type. However, the IBV genome consists of single stranded RNA with a high mutation frequency. Molecular studies with IBV have shown that new IBV serotypes and genotypes can emerge as a result of only a very few changes or mutations in the amino acid sequence of the spike gene, while the major part of the virus genome remains unaltered. Also, several workers demonstrated or provided circumstantial evidence that IBV can undergo recombination during mixed infections. As a result, IBV strains may show multiple cross-reac-

tions, making a clear classification not always possible.

Classification systems are divided into two major groups: functional tests, that regard the biological function of a virus, and non-functional tests that look at the viral genome. Typing by functional tests results in immunotypes or protectotypes, and antigenic (serotype or epitope) types. Tests that look at the genome result in genotypes. The preferred typing system depends on the goal (e.g. selection of vaccination programmes, or epidemiology), available techniques, experience and costs.

Immunotypes or protectotypes

Grouping of IBV strains into immunotypes (Cunningham, 1975) or protectotypes (Lohr, 1988; Hinze, et al., 1991) is the most important system from a practical point of view, because it provides direct information about the efficacy of a vaccine. Strains that induce protection against each other, belong to the same immuno- or protectotype. The number of protectotypes that exists is unknown, but cross-challenge experiments in chicken tend to reduce the number of protectotypes types compared to serotypes, presumably because they are measuring the complete immune response and not just a part of it (McMartin, 1993).

To determine the protectotype of a strain, a cross-immunisation study (CIS) has to be performed. A CIS is labour-intensive, expensive and requires many animals and isolation facilities. A more economical in vitro alternative for the CIS may be the cross-immunisation test (CIT). In a CIS, vaccinated birds are challenged in different groups with different strains. When performing a CIT, the challenge of vaccinated chickens is performed on TOCs of those birds, testing the tracheal cross-immunity. Because one bird provides many tracheal rings, each bird can be challenged with a number of different isolates. Although TOCs simulate the chicken better than most in vitro methods, there might be differences. When the challenge is done in chickens, the whole immune system is available, whereas in TOCs, this is probably not the case. Whether CIT is really a reliable alternative for the CIS needs further work, preferably in comparative studies using different strains of different tropism.

Antigenic types

Serotypes

The classical functional typing system is serotyping by VN-test, which is based on the reaction between an IBV strain and chicken-induced IBV serotype-specific antibodies.

A disadvantage of serotyping is the lack of standardisation between the different systems and users. The results of different laboratories are therefore not always comparable.

Epitope types (monoclonal antibody)

By incorporating a Mab into the test, it is possible to check whether a certain epitope or a number of epitopes is present on a strain. When this (these) epitope(s) is (are) related to a certain serotype, it can be used to produce a serotype-specific test such as antigen-ELISAs or IFAs. Use of serotype-specific Mabs, which are directed against hypervariable parts of the S1 protein, has a certain risk of false-negative results. A mutation in

the epitope to which the Mab is directed, does not necessarily mean that the strain has changed to another serotype. So, an IBV virus that does not react with a panel of serotype-specific Mabs is preferably re-tested by a different technique to check whether another serotype is involved, or whether possibly one of the epitopes was changed by mutation and therefore missed by the panel of Mabs that was used.

Genotypes

Grouping of strains based on genetic characterisation of (a part of) the genome (or its c-DNA) results in genotypes. Methods include sequencing part(s) of the genome or determining the position of enzyme cleavage sites (RFLP, RNase T1 fingerprinting). Genomic information is objective and provides essential information for epidemiological studies. A disadvantage of genotyping for use in the field, is that direct translation of information about a part of the genome of an IBV strain to biological function or antigenicity of the virus is not possible or not without risk. Isolates of the same serotype or protectotype can differ substantially in some genes (Cavanagh et al., 1992a), while different serotypes or protectotypes can have remarkably high similarity between their genomes (Clewley et al., 1981, Kusters et al., 1987; Williams et al., 1992; Cavanagh et al., 1992a, 1992b). Although high correlations between the genotype and serotype of strains have been reported, other papers present conflicting data about strains genotype and serotype or even protectotype.

Therefore, for practical use in the field, exclusive use of genotyping methods is not recommended. Especially when there is suspicion in the field that the genotype of recent isolates does not provide accurate information about the true antigenic nature of IBV isolates, conventional testing (serotyping) and especially in vivo studies are required.

a: sequencing

Sequencing and subsequent comparison of the amino acid sequences of viral proteins is a very useful instrument to help locating conserved domains in proteins that might be essential for their structure and function and for epidemiological studies. Based on sequence data, a phylogenic tree can be made, revealing the genomic relatedness between different strains. However, it must be remembered that the place of a certain strain in a phylogenic tree can differ depending on the genotyping techniques used, or on which part of the genome is analysed. Sequence data only provide information about the primary structure (sequence of amino acids) of the protein. Detected differences in sequence of two strains can not be translated to differences in antigenity or biological function. Also the occurrence of recombinations between different IBV strains hampers the translation of data of a genotype to a serotype or protectotype.

b: restriction enzyme fragment length polymorphism (RFLP)

After amplification of cDNA of the S1 gene by PCR and purification by electrophoresis, the PCR product is digested with restriction enzymes that cut cDNA into fragments at certain highly specific cleavage sites. The RFLP patterns are then compared with patterns of representatives of known serotypes. This test can provide a fast diagnosis, including typing of the virus. However, cleavage sites of enzymes used in RFLP are not related and cannot be translated to biological or antigenic func-

tion of the virus. The correlation between RFLP pattern and serotype can be high, but as reported by Hein et al. (1998), different isolates, typed by RFLP as belonging to the same genotype, can be of different serotypes or protectotypes. Therefore, it is recommended that where there is suspicion in the field that the RFLP-genotype of recent isolates does not provide accurate information about the true antigenic nature of IBV isolates, conventional testing and especially in vivo studies are also used.

c: RNase T1 fingerprinting analysis

After multiplication of IBV, extracted and purified viral RNA is digested with ribonuclease (RNase) T1, resulting in oligonucleotides. After staining, the oligonucleotides are resolved on two-dimensional gel electrophoresis, resulting in a fingerprint of the genome. The fingerprint can be compared with fingerprints of known strains. This complex and labour-intensive technique is hardly used anymore for IBV because it does not work well for genomes with less than 95 % homology (what is not unusual for IBV) and shows poor correlation between genotype and serotype.

Detection of antibodies

IBV infections can be diagnosed by detecting the appearance of, or rise in, antibody titre of IBV specific antibodies. Generally, in order to be able to correlate a clinical problem with an IBV infection, paired sampling is required. The first sample is taken at onset of disease, and the second sampling several weeks later. The paired sera should be tested in the same test run, to prevent wrong conclusions caused by day-to-day variation of the tests. At least a four-fold rise in titre is required for a positive diagnosis.

Factors influencing the success of IBV antibody detection

Interpretation of serological results can be complicated by a number of factors including presence of immunity at time of vaccination/infection, cross-reactions between serotypes, and occurrence of new or unexpected IBV strains. Furthermore, lack of attempts to standardise tests between different laboratories adds to the difficulty in interpreting results.

Presence of immunity at time of vaccination/infection

A very important factor that influences the degree of humoral response after an infection or vaccination is the immunity derived from previous vaccination or infection (Gazdzinski et al., 1977; Macdonald et al., 1981; Darbyshire & Peters, 1984, 1985; De Wit et al., 1997). The degree of humoral response after infection in vaccinated chickens can be decreased and slowed down compared to the humoral response of unprotected birds (Figure 3 and 4). As a result, the sensitivity of antibody detecting tests can be much lower in vaccinated chickens as compared to unvaccinated chickens.

Cross-reactions between serotypes

A complicating factor in serotyping an IBV infection by serology is the cross reaction which may occur after an infection. These cross reactions especially occur in birds that had multiple contacts (e.g. vaccinations) with IBV (Table 1), particularly when more than one serotype is involved (Gelb & Killian, 1987; Karaca & Naqi, 1993; De

Figure 3: Average HI and ELISA titre after D8880 infection of unvaccinated broilers
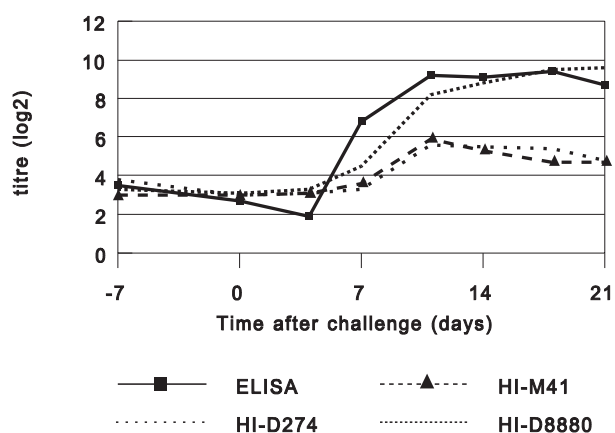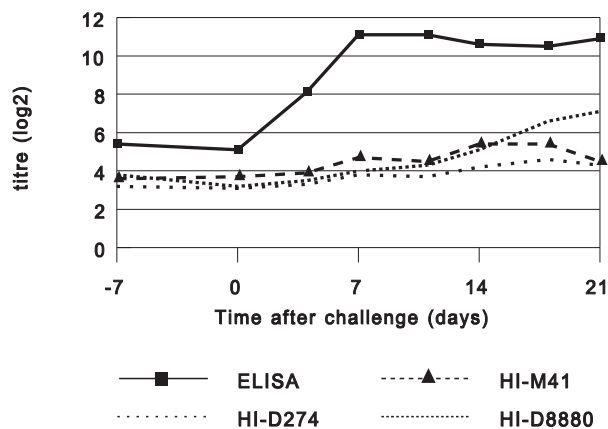

ELISA — HI-M41
HI-D274 — HI-D8880

Figure 4: Average HI and ELISA titre after D8880 infection of H120-vaccinated broilers

ELISA — HI-M41
HI-D274 — HI-D8880

Wit et al., 1997).

For interpreting serological results, these heterologous cross reactions in serotype-specific tests have to be differentiated from the homologous response. When the cross reactions are high, as may be expected in older birds that have been vaccinated (and probably infected) several times with different serotypes, interpretation of the serological data can be very difficult or even impossible. In those conditions, subjectivity of the interpreting person is a real danger for the quality of the diagnosis. Obviously, a particular IB-serotype can only be identified if that serotype is included in the panel of IBVs tested. If not, the highest cross reaction may be misinterpreted as the serotype involved.

Occurrence of new or unexpected IBV strains

When serology is used for serotyping the IBV infection, a selection of serotype-specific tests will be requested, based on experience, tradition or availability. Not-requested serotypes, like new or unexpected serotypes, will not be included in the requested panel of serotype-specific tests. Under these circumstances, there is a high risk of wrongly appointing the serotype with the highest cross reactions as being the serotype involved in the infection. Therefore, to minimise this risk of missing the correct serotype, serotype-specific tests for all known serotypes (based on typing of isolates) circulating in the "region" should be used. It is important always to remember this limitation of serotype specific diagnostic tests.

Tests for antibody detection can be grouped into group-specific and serotype-specific tests.

Group-specific tests

Group-specific tests for detection of antibodies against IBV do not differentiate between antibodies induced by strains of different serotypes. Although the antigen that is used in the test is of one serotype (usually Massachusetts), it also detects antibodies against other serotypes. The AGPT and ELISA are such tests.

Table 1: Mean VN titres after an IBV infection in non-vaccinated and vaccinated broilers (Avian Pathology,

Group	Vaccination with H120 at day 1	Challange with IBV at day 28	Mean VN titre[a]							
			M41		D274		D1466		D8880	
			day		day		day		day	
			28	49	28	49	28	49	28	49
A1	-	-	-[b]	-	-			-	-	-
B1	-	-	-	-	-	-	-	-	-	-
A2	-	M41	-	7.6	-	-	-	-	-	-
B2	-	M41	-	8.3	-	-	-	-	-	-
B5	-	D274	-	-	-	7.1	-	-	-	-
B6	-	D1466	-	-	-	-	-	9.5	-	-
B7	-	D8880	-	-	-	-	-	-	-	9.9
A3	yes	-	4.1	4.9	-	-	-	-	-	-
A3	yes	-	4.0	4.8	-	-	-	-	-	-
A4	yes	M41	4.4	7.6	-	-	-	-	-	4.0
B4	yes	M41	4.3	6.9	-	-	-	-	-	-
A5	yes	D274	4.4	6.7	-	6.7	-	-	-	5.5
A6	yes	D1466	-	5.1	-	-	-	5.3	-	4.1
A7	yes	D8880	4.6	6.6	-	5.8	-	-	-	8.1

[a] VN titres expressed as $\log_2$ of the reciprocal of the highest serum dilution that showed complete inhibition of CPE.

[b] VN titre <4

Agar gel precipitation test (AGPT)

Performing the group-specific AGPT requires almost no laboratory facilities. With this test, inclusion of a positive control serum adjacent to the well with test serum is important to be able to differentiate non-specific precipitation bands from IBV-specific bands (Woernle, 1966). Because of its ability to differentiate non-specific from specific reactions, the AGPT has a high specificity, although the precipitation lines for IBV are more difficult to read then for other agents like Gumboro or Newcastle virus. Although the test is relatively simple, there is an absolute lack of standardisation of the test for IBV. Precipitins are usually detected about a week post-infection or vaccination, usually followed by a fast decrease of percentage of positive samples a few weeks later, although extended periods are reported. Because of the transient reactions of the AGPT, positive results indicate a recent contact with IBV.

The reported sensitivity of the AGPT varies from 0 to 100 %, depending on the IBV strain inoculated, application route, age of infection, presence of MDA or vaccinal immunity at the time of challenge, and test performance. Therefore, use of a non-validated AGPT is not recommended.

Group-specific ELISA

Reports of indirect ELISAs show them to be group-specific. Antibodies can first be detected by ELISA within a week after vaccination or infection. Because of the short period between infection and the detection of the first antibodies by ELISA, the first of paired sampling must be done at the first signs of IBV, which usually appear between 18 and 36 h after infection. If the first sampling is not done in time, seroconversion can be missed.

α-IBV-IgM ELISA

IBV-specific IgM is only present temporarily after an infection or vaccination. Therefore, it's detection is indicative for a recent infection or vaccination. However, the reports regarding IgM detection after IBV vaccination and infection are limited and partly conflicting. Therefore, more data collected under different conditions are needed before IgM detection techniques are suitable for use in the field.

Serotype-specific tests

Serotype-specific tests for detection of antibodies against IBV differentiate between antibodies induced by strains of different serotypes.

Virus Neutralisation test (VNT)

The VNT is the gold standard test for the detection of IBV serotype-specific antibodies. The specificity is very high after a single IBV inoculation (Table 1). Cross-reactions can occur after multiple contacts with different IBV serotypes (Gelb & Killian, 1987; De Wit et al., 1997), although some studies also report low cross reactions after repeated inoculations with the same serotypes (Karaca & Naqi, 1993).

Haemagglutination inhibition (HI) test

The first haemagglutination inhibiting antibodies are usually detected between 1 and 2 weeks after infection.

The HI-test is like the VNT a serotype-specific test when used to detect antibodies after a single inoculation with IBV. Although the correlation between both test systems is high under these circumstances, the specificity of the HI is considered to be lower than that of the VNT (King & Hopkins, 1983; Gelb & Killian, 1987; De Wit et al., 1997).

The serotype-specificity of the HI-test is much lower following re-infection with IBV, especially when the second or subsequent serotype is heterologous (King & Hopkins, 1983; Gelb & Killian, 1987; Brown & Bracewell, 1988; De Wit et al., 1997). Sequential inoculation with IBV can induce antibodies that react in HI test with serotypes to which the birds had not been exposed.

Serotype-specific ELISA

A new approach of producing serotype-specific tests was tried by Karaca & Naqi (1993) by using serotype-specific Mabs as blocking agent in two blocking ELISAs. Whereas the results after primary inoculation seemed to be serotype-specific, heterologous cross reactions were detected by both the blocking ELISAs after a secondary inoculation with the homologous virus.

Relative sensitivities of AGPT, ELISA, HI-test and VNT

Many studies have compared the performance of two or more serological tests. Due to the many variations in experimental conditions, test performance and kind of antibodies that are detected by the different tests, different results are reported. But generally, antibodies can first be detected by ELISA, followed by AGPT and HI-test, and last by VNT.

Last recommendations for use of serology

The data of all serotype-specific antibody tests show that the presence of antibodies against a certain serotype in vaccinated chickens is not necessarily a proof of an infection caused by that serotype. Therefore, one should be very cautious in concluding that a new serotype is present in a region, only on the basis of detecting antibodies by a serotype-specific test in sera of vaccinated chickens. Detection of the virus itself is more reliable. When serology is used for screening a region for the presence of a certain serotype of IBV, testing by VNT of sera of unvaccinated chickens is preferred.

Serology is better suited to monitor the presence of already known serotypes (clinical or subclinical infections), especially in younger birds. When group specific tests like AGPT or ELISA detect IBV infections without clear titres in serotype-specific tests, possibly a new serotype has arrived. For older birds that have been vaccinated (and maybe infected) several times using vaccines of different serotypes, serology is far better suited to monitor the presence of IBV infections in general than being able to serotype them. Again, detection and typing of the virus itself is preferred in these circumstances.

Use of serology to check the take of vaccines often requires very sensitive tests that are properly validated for that purpose. Lack of detectable amounts of vaccine-induced antibodies do not prove that their is no vaccine-induced protection, as this can be local protection.

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