THE DIAGNOSIS AND CONTROL OF INFECTIOUS BRONCHITIS
VARIANT INFECTIONS

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ABSTRACT

In this paper the diagnosis of IB infections is discussed. This is based on clinical signs and laboratory examinations. The laboratory tests can be divided into 2 groups.

A. Serological tests

- agar gel precipitation test,
- virus neutralization test,
- haemagglutination inhibition test.

B. Demonstration of viral antigen

- immunofluorescence test,
- virus isolation and identification.

Using these techniques the following programme is used to control IB:

1. Inventory of the incidence of IB infections.
2. Virus isolation and serotyping; monitoring of incidence.
3. Development of vaccines.
4. Evaluation of the vaccination programme.

Implementation of this programme has resulted in a sharp decrease of IB infections in The Netherlands.

INTRODUCTION

Despite vaccination against Infectious Bronchitis virus (IBV), infection of poultry with this virus still causes disease problems in many countries. Entitled with the common name Infectious Bronchitis (IB), disease symptoms vary greatly from respiratory distress in young birds to a peak of egg production below predicted levels, depressed production, shell abnormalities and intercurrent diarrhoea in laying and parent stock or a combination of these symptoms.

In the Netherlands IB is controlled mainly by vaccination with the live attenuated vaccine viruses H120 and H52. Since 1976 vaccination with the more attenuated H120 vaccine virus has been carried out mainly by application as a coarse droplet spray to newly hatched chicks. The less embryo-adapted H52 vaccine virus is subsequently applied, preferably by eye drop, at an age of about 15 to 18 weeks. The immunological basis for the day-old spray application has been published before (Davelaar et al., 1982).

The H120 and H52 vaccines based on the H-strain originally isolated

by Bijlenga et al. (1956) are up to the present the best option for a vaccine against IB since these vaccines have a wide antigenic spectrum. Winterfield (1975) showed that many, although not all, of the IBV strains isolated in the USA from birds suffering from respiratory and/or production problems (these strains to be distinguished from those causing kidney problems, such as the Holte, Gray and Australian T strains), were immunologically "covered" well by the H-vaccine.

It was therefore surprising that the improved results achieved by the newly introduced improved vaccinations were not consistent (text fig. 3).

From 1979 an increasing number of properly vaccinated flocks suffered from respiratory (mainly broilers) and from egg production problems as described. In many of these cases IB was diagnosed by a combination of serology (rise in HI titers and numbers of birds reacting in the AGP test), demonstration of IBV antigen with the direct immunofluorescence test (IFT) in frozen tracheal sections and virus isolation.

In this paper the significance of clinical observation and of laboratory tests to diagnose IB will be discussed. Thereafter the design and the results of our study on the situation in the Netherlands are presented.

DIAGNOSIS

Clinical signs

In practice IB in young chicks is diagnosed on respiratory signs. In adult birds IB problems are manifested by either dramatically decreased egg production with typical egg shell problems and occasionally respiratory problems or by failure to reach peak production or by depressed production, or by a combination of the signs. In many cases however, due to a lack of obvious signs, there is a need for a definitive diagnosis by laboratory tests. These tests are based on the demonstration of antibody (serology) or on the presence of the viral antigen (immunofluorescence and isolation).

Serological tests

- The agargel precipitation (AGP) test (Woernle, 1959).

With this type of test precipitating antibodies can be demonstrated in blood or serum. A couple of weeks after infection the concentration of precipitating antibodies reaches its peak and declines thereafter. In other words, shortly after infection a high percentage of AGP positive sera will be found, whereas about one month later a clear decline in the

number of positive reactors appears. In the test the group antigen of the avian coronaviruses is detected and the test is therefore not serotype specific.

- Virus neutralization test.

The virus neutralization (VN) test in embryonated SPF eggs (alpha method) used to classify IBV isolates and to detect antibody in poultry flocks has been described by Lukert (1975). The test is serotype specific.

- Haemagglutination inhibition test (HI-test) (Alexander and Chettle, 1977).

IBV is able, after treatment with the enzyme phospholipase - C, to bind with erythrocytes (= haemagglutination). By incubating the virus with antiserum, the haemagglutination can be blocked. By testing antisera in twofold dilutions against the haemagglutination inhibition ability, the antibody-titer can be determined.

We have demonstrated that the HI test is serotype-specific.

Text figure 1 shows that the homologous HI titer after a single eye drop inoculation of chicks with the D274 isolate is high after 2

TEXT FIGURE 1

HI
(Log base 2)

weeks and persists at the same level until the end of the experiment. HI titers measured with antigens of the Massachusetts and the D1466 serotypes peak 2 weeks after inoculation and decline thereafter. So group-specific reactions occur only for a short time after inoculation. Table 1 represents the results of cross HI tests carried out with sera obtained 5 weeks after inoculation. The results show the relative type-specificity of the HI tests.

TABLE 1 HI experiment demonstrating the type difference between the new isolates (Titer expressed as $\log_2$).

Serum \ Virus (Type)	M41 (Mass)	D274 (207)	D1466 (212)
H52 (Mass)	9	6	5
207	6	10	5
212	5	5	8
246G (Mass)	10	5	n.d.

Demonstration of viral antigen

- Immunofluorescence test (IFT).

With this technique IBV can be visualized in cryostat sections by reacting the virus antigens with fluorescent antibody (conjugate). Usually IBV can be demonstrated in the trachea up to 10 to 14 days after infection. The test is not serotype specific but reacts with the group antigen of the avian coronaviruses.

- Virus isolation and identification.

In our study IBV was primarily isolated from tracheal samples, even in cases of egg production problems without evident respiratory symptoms. In a later stage of the study we found that including the upper part of the caeca containing the caecal tonsils increased the isolation scores greatly.

However, a comparison between isolation from trachea and caeca has not been carried out. Freshly collected tracheal samples (appr. 3 cm long) from 3 to 5 birds from an affected flock were cut in longitudinal slices and ground together with the upper caeca from the same birds in a mortar and pestle with sterile sand after addition of cold sterile saline to make

up an organ suspension of about 10 volume per cent. Tracheas are not completely macerated by this treatment but it yields a suspension of sufficient mucous membrane in saline. After grinding, the suspension was centrifuged for 30 mins at 3000 g and 4°C. The supernatant was incubated for 30 mins at 4°C with 5 mg Streptomycin and 5000 IU Penicillin per ml and then 0.2 - 1 ml was inoculated into the allantoic cavity of each of 5, 8-10 days embryonated eggs from a specific pathogen free (SPF) flock. Eggs were candled daily. Embryo mortality within 24 hours after inoculation was regarded as non-specific.

When embryo mortality or embryonic changes (dwarfing, clubbed down) had not occurred by day 6 after inoculation, eggs were cooled overnight at 4°C. One ml volumes allantoic fluid were collected from each embryo the next day, pooled, incubated with antibiotics and inoculated into the allantoic cavity of five other embryos as before. This procedure was repeated three more times (total 5 embryo passages of the suspected suspension) before a sample was regarded as negative. It should be remarked that in some exceptional cases up to 12 passages had to be made before a virus was isolated.

When embryo changes or embryo mortality was observed, the chorio-allantoic membranes (CAM) of the infected embryos were collected, blotted with tissue and small pieces were minced and examined in an AGP test with monospecific precipitating fowl antisera against IBV, REO virus, Adenovirus, Infectious bursal disease virus, Infectious laryngotracheitis virus, avian influenza, Newcastle disease virus and fowl pox. Essentially the AGP test was carried out as described by Woernle (1959). Gels were incubated for 24 hours at 37°C.

When both the IFT and AGP test failed to confirm the identity of the isolate, IBV antiserum was used to inhibit the potency of the virus infected allantoic fluid to induce embryo mortality or embryo changes. Initially only antiserum against the H-vaccine type was used. Later antisera against this and the four novel serotypes described in this paper were available. The hyperimmune sera were mixed in equal volumes and the mixture diluted 1:20 with phosphate buffered saline (PBS). Of this mixture 9 volumes were mixed with 1 volume allantoic fluid and incubated for 30 mins at 40°C before injection into embryos as before.

PROGRAMME FOR THE CONTROL OF IB

The investigations performed to diminish IBV infections in the Netherlands can be summarized in the following design:

Stage 1 - Inventory of the incidence of IBV infections

- a. Clinical signs
- b. Serology - AGP
- c. IFT

Stage 2 - a. Virus-isolation and serotyping (Cross VN tests)

- b. Serology - check on incidence of antibody against these serotypes in the field (HI; VN)

Stage 3 - Development of vaccines against the predominant serotypes

Stage 4 - Evaluation of the vaccination programme by monitoring flocks

- a. Clinical signs
- b. Serology (seroresponse)
- c. Problem flocks

Be aware of novel serotypes

stage 2

RESULTS

Stage 1

A. An example of a flock with egg production problems due to IBV is given in text figure 2. In the figure the percentage of sera from 24 birds reacting positively in the AGP is also expressed. The AGP-curve is in strict harmony with the overall depressed production and with the drop in egg production later on.

B. Results of the IB diagnosis by IFT in the period 1974-1983 are presented in text figure 3. As demonstrated in the figure outbreaks of IB were observed regularly in 1974. Therefore an investigation was started to devise a better programme for IB vaccination of broilers. As a result of this study, vaccination of day-old chicks in the hatchery was introduced, resulting in a sharp decrease in IB problems. During 1976-1977 IB was diagnosed frequently in laying hens. Vaccination with the H52 vaccine by eye drop resulted in a low incidence of egg problems in breeders and layers in 1978-1979. However in 1980 there was an increase in IB problems in laying hens with dramatically decreased egg production in conjunction with typical

TEXT FIGURE 2

TEXT FIGURE 3

TABLE 2 Survey of IBV isolation trials of 992 flocks suspected of Infectious Bronchitis.

Year	Isolations	Serology													
		D207		D212		D274		D3128		D3896		H52		Other	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
1979	39/167*	19/ 30**	63	4/13	13	-	-	-	-	14/ 31	45	7/ 33	21	2/ 33	6
1980	48/302	13/ 26	50	2/26	8	12/20	60	5/21	24	12/ 26	46	4/ 26	15	1/ 26	4
1981	34/285	6/ 29	21	6/29	21	9/29	31	7/29	24	5/ 29	17	11/ 29	38	1/ 29	3
1982	41/328	2/ 17	12	1/17	6	3/17	18	6/27	35	1/ 17	6	13/ 17	76	2/ 17	12
Total	162/992	40/102	39	13/85	15	24/66	36	18/67	27	32/103	31	35/105	33	6/105	6

* numbers of isolates / numbers of trials to isolate IBV

** number of isolates corresponding with this serotype / number of isolates tested

TABLE 3 Reciprocal cross neutralization test of American strains, Holland 52 Massachusetts type strain and primary IBV isolation of 1978 and 1979.

Virus / serum	I 97	I 609	Connecticut	Florida	JMK	3896	207	212	H52 (Mass.)	3128
I 97	≥6.2	0.7	1.2	1.4	0.7	1.7	0.0	0.0	0.7	1.9
I 609	≤2.9	4.9	≤2.9	≤2.9	≤2.9	3.6	≤2.9	≤2.9	≤5.1	≤3.1
Connecticut	2.1	2.1	≥6.8	6.1	1.8	3.6	1.6	≤1.2	4.1	≤2.6
Florida	0.6	1.3	3.4	≥4.2	0.5	2.6	1.0	0.0	1.3	4.5
JMK	2.8	1.5	1.4	2.3	≥6.3	2.8	1.3	1.0	1.2	4.5
D3896	≤0.8	0.9	1.5	1.5	≤0.8	≥6.6	1.9	≤0.3	≤0.7	3.2
D207	0.5	0.0	0.4	0.5	≤0.3	2.6	≥5.3	1.3	1.4	1.4
D212	0.0	1.2	1.0	1.0	0.3	2.3	0.5	5.3	0.5	≤1.4
H52	1.6	2.8	1.8	2.8	1.1	1.8	1.1	1.1	≤5.3	3.7
D274	2.2	2.7	1.9	3.7	2.0	4.2	5.0	≤1.5	≥1.6	3.4
D1466	ND	ND	ND	ND	ND	1.8	1.2	≥4.9	0.5	3.1
D3128	1.1	2.1	2.8	3.3	1.3	1.5	0.0	0.0	0.0	≥6.4
D752	1.7	1.7	3.0	1.2	1.9	1.9	1.5	1.3	1.5	1.9

ND = not done

egg shell problems. In most cases IB virus could be demonstrated in the trachea by means of the IFT. In 1982 and 1983 there was a striking decrease in the incidence of IB. This was effected by a radical change in the IB vaccination programs of laying and breeder flocks (see later).

Stage 2

A. Virus isolation and identification. IBV was isolated from 162 of 992 IB suspected flocks during the period 1979–1982 (Davelaar, 1984). This means that about 16 per cent of the isolation attempts were successful (Table 2). In reciprocal cross-neutralization tests with the American strains Iowa 97, Iowa 609, Connecticut, Florida, JMK and the Massachusetts type vaccine strain H52, the majority of the primary isolates could be classified into 4 major serogroups that differ from the American strains and the H52 strain (Table 3). The new serogroups are called D207, D212, D3896 and D3128 (D = Doorn, the number stands for the laboratory number of the first isolate). Notice that isolate D274 is closely related to the serogroups D207 and D3896 (Table 3). Based on the definition that strains with NI values ≥ 4 in cross-neutralization tests with known antisera are considered to belong to the same serogroup, isolate D274 is classified in both serogroups D207 and D3896. Further investigations showed that a majority of isolates corresponding to isolate D274 ($NI \geq 4$) are also related with the serogroups D207 and D3896. Table 2 shows a survey of isolates obtained and characterized during the years 1979–1982. In the years 1979 and 1980 viruses of the serotypes D207 and D3896 were predominant, thereafter the number of isolates of these serotypes decreased in favour of the Massachusetts type viruses.

B. Serological survey. The results of a survey of 328 flocks for the presence of antibody against the IBV serotypes D207, D212, D3896 and D3128 are listed in Table 4. This survey shows a distribution of antibody in

TABLE 4 Frequency of IBV variant types in The Netherlands.
Serological examination of 328 flocks.

Strain (type)	D207	D212	D3128	D3896
Flocks monitored				
No. 328	214	107	180	218
Percentage	65	33	55	66

flocks comparable with the isolation pattern. Antibody to the serotypes

TABLE 5 Ten groups of 12 weeks old chicks (each group 5 birds) were eye drop vaccinated with H120 vaccine strain and challenged by the same route 4 weeks later.

Group	Experimental design		Histology of the trachea			Virus recovery	
	Vaccinated with H120	Challenge	pre chall.	4 days after	10 days after	total lesion score	4 days after chall.
1	no	D387		1.8*	1.0*	1.4**	3/5***
2.	no	D207		1.7	1.2	1.4	4/5
3.	no	D212	0*	1.0	0.3	0.7	3/5
4.	no	D3128		2	1.0	1.5	3/5
5.	no	D2896		1.5	1.0	1.3	3/5
6.	yes	D387		0	0.2	0.1	0/5
7.	yes	D207		0.5	0.7	0.6	3/5
8.	yes	D212	0	0.3	0.3	0.3	2/5
9.	yes	D3128		1	0.5	0.8	2/5
10.	yes	D3896		0.9	0.8	0.8	1/5

* Mean lesion score of 6 tracheas per group

** Mean lesion score of 12 tracheas per group collected at 4 and 10 days after challenge

*** Number of embryonated eggs showing abnormalities typical for IBV / number of eggs inoculated with tracheal scrapings.

D207, D3896 and D3128 was observed in 55-66 per cent of the flocks, while antibodies against serotype D212 were present in only 33 per cent of the flocks.

Stage 3

A. Cross-immunity tests in chicks. Cross-immunity tests in chicks were carried out to examine whether vaccination with a Massachusetts type vaccine (H120) would provide protection against the novel serotypes. The results of a challenge experiment are summarized in Table 5. Vaccination with H120 protected the birds against tracheal lesions caused by the Massachusetts type challenge virus (D387), but was not able to reduce the lesions caused by the other strains for more than 50 per cent. The results of the virus recovery test confirmed the histological findings. VI was negative only in group 6, vaccinated with H120 and challenged with Massachusetts type virus. In contrast VI was positive in the H120 vaccinated groups challenged with heterologous viruses and in the non vaccinated challenged chicks. It appears from the lesion scores in Table 5 that the isolates D207, D3896, D3128 and D387 have similar pathogenic properties to the trachea, while the pathogenic effect of isolate D212 is much more limited.

B. Table 6 illustrates the various approaches to control IB by vaccination. We focussed our activities primarily on the search for a virus strain with good immunogenic properties and a broad spectrum. The isolate D274 belonging to the serotype D207 induced cross-protection against D207, D3896 and to a lesser extent against D3128, but no protection against D212 challenge. During further investigation of that vaccine candidate strain, D274, we found out that D274 virus agglutinated chicken red blood cells spontaneously. The agglutination was observed with virus preparations consisting of untreated allantoic fluid harvested 24-72 hours p.i. and with supernatant from infected CEK cell cultures.

TABLE 6 Control of IB by vaccination

Monovalent live vaccine	→	Preferably broad spectrum strains H-strains; strain D274
Polyvalent live vaccine	→	Risk of interference
Inactivated vaccine	→	Polyvalent; use restricted to breeders and layers

SPF birds vaccinated with attenuated (by embryo passage) D274 strains responded with extremely high HI and neutralization antibody titers. The haemagglutinating (HA⁺) strain of D274 appeared to be more immunogenic than the non haemagglutinating (HA⁻) strains belonging to the same serotype (e. g. isolate D207) (Table 7) (Davelaar, 1983). This observation was confirmed by a series of laboratory experiments which encouraged us to use D274 as a vaccine strain in the field. Spontaneously haemagglutinating strains have since been found among other serotypes and we have some experimental evidence that the immunizing potency of IBV strains showing spontaneous haemagglutination of chicken red blood cells, exceeds the potency of virus strains belonging to the same respective serotypes but which do not spontaneously agglutinate RBC's. Chickens were vaccinated with 10⁵ EID₅₀ per bird

TABLE 7 Mean HI titer of ten vaccinated hens per group.
HI antigen prepared with IBV D274.

Vaccine	Weeks after vaccination											
	0	1	2	3	4	6	7	8	9	10	11	12
207 (HA ⁻)	4.0	5.4	6.2	5.9	6.6	4.8	4.2	5.5	5.8	5.2	4.1	5.2
274 (HA ⁺)	4.7	7.5	8.2	8.7	8.2	10.0	9.3	10.0	10.1	9.5	8.8	9.3
none					←	< 5		→				

by eye drop with experimental vaccines prepared from the HA⁻ strains D207 and the HA⁺ strains D274 respectively. HI antibody response was measured in weekly intervals, the sera were tested for VN AB's at 3 and 12 weeks p. v. and the birds were subjected to a challenge infection with a low egg passage D207 strain 12 weeks p.v. Table 7 shows results of HI testing, peak mean titer 6.6↔10.0 and 12 weeks p.v. still 9↔5 in the D207 group. Table 8 demonstrates that the VN response was better in the D274 group 12 weeks p.v.: 9/10 birds had a specific protective titer, 5/10 in the D207 group. The same applies for challenge: complete protection in D274 group- 60% in D207 group against the homologous challenge virus (Table 9). Table 10 illustrates that after combination of two HA⁺ strains the immune response is significantly better than in a combined live vaccine consisting of two HA⁻ strains.

Stage 4

A. Since 1982 we have been working in the field with vaccination pro-

TABLE 8 *Number of animals (out of ten vaccinated) showing a neutralizing response > 4.0 log against the homologous strains

Weeks after vaccination	Vaccine group	
	207 (HA ⁻)	274 (HA ⁺)
3	7/10*	9/10*
12	5/10	9/10

TABLE 9 *Number of birds (out of ten vaccinated) showing microscopical lesions or positive IFT reactions in the trachea 4 days after challenge infection.

Vaccine	Microscopic lesions	IFT positive
207 (HA ⁻)	6/10*	2/10
274 (HA ⁺)	0/10	0/10
non	10/10	6/10

grammes based on live IB vaccines (Table 11) alone or in combination with inactivated vaccines (Table 12). Using these vaccination programmes based on HA⁺ isolates the incidence of IB decreased from approximately 12 per cent to less than 4 per cent within a period of 2 years (text figure 3). The strains used for these vaccinations and their relation to the Dutch serotypes are expressed in Table 13.

B. Problem flocks. IBV is isolated from flocks suffering from IB. Changes in the pattern of isolations during the period 1980-1985 are presented in Table 14.

TABLE 10 HI response* after vaccination with different live IB vaccines of D207 serotype.

Vaccine	Mean HI titer of ten birds* weeks after eye drop vaccination				
	0	2	4	6	8
D274 (HA ⁺)	4.0	9.8	10.0	10.0	10.3
D274 + D1466 (HA ⁺)	3.9	8.0	9.0	8.4	9.0
Commercial combined					
D207 + D212 (HA ⁻)	3.9	6.2	6.6	5.2	5.9
Controls	3.8	4.0	3.9	3.6	4.2

*HI test against serotype D207

TABLE 11 Vaccination program of laying and breeder flocks based on live vaccines

1 day	Mareks disease	Injection at hatchery
1 day	IB, H120	Spray at hatchery
1 week	ND, H or C	Knap sack
2-3 weeks	Gumboro disease	Drinking water
5 weeks	ND, H C or L	Knap sack or Atomist
8-10 weeks	IB, D1466	Eye drop or knap sack
10-12 weeks	IB, D274	Knap sack (or eye drop)
12-14 weeks	Epidemic tremor	Oral or spray or drinking water
	ILT	Eye drop
	Fowl pox	Wing web
14-16 weeks	IB, H52	Eye drop or knap sack
16-20 weeks	ND, C or L	Atomist

ND = Newcastle disease
 H = Hitchner
 C = Clone 30 (Intervet ND vaccines)
 L = LaSota strain of ND
 ILT = Infectious Laryno Tracheitis

TABLE 12 Vaccination program for laying and breeder flocks based on live and inactivated vaccines.

1 day	Mareks disease	Injection at hatchery
1 day	IB, H120	Spray at hatchery
1 week	ND, H or C	Knap sack
2-3 weeks	Gumboro disease	Drinking water
5 weeks	ND, H, C or L	Knap sack or Atomist
8-10 weeks	IB, D1466	Eye drop or knap sack
10-12 weeks	IB, D274	Knap sack (or eye drop)
12-14 weeks	Epidemic tremor	Oral or spray or drinking water
	ILT	Eye drop
	Fowl pox	Wing web
16-20 weeks	IB, H' + variant strains	Injection
	ND	Injection
	Gumboro disease	Injection reproduction
	EDS	Injection, infected farms

Explanation see Table 11

Variant strains = D274 + D1466

H' = Massachusetts type strain M41

EDS = Egg drop syndrome

In 1980-1982 about 90 per cent of the isolates (79) were classified into one of the 4 serogroups (NI ≥ 4). In contrast in 1984-1985 37 per cent of the isolates (110) did not belong to one of these serogroups. They showed

TABLE 13 IBV serotype, their HI antigens and vaccine strains.

Serotype	Mass.	D207	D3895	D3128	D212
HI-antigen	M41	D274 (HA ⁺)			D1466 (HA ⁺)
Vaccine strains	H120 H52 M41	D274 (HA ⁺)			D1466 (HA ⁺)

weak to moderate cross-reactions with the serotypes D1466, D3128 and H52. This suggests that the highly immunogenic vaccine strain D274 prevents mutation of viruses of the same serotype. Other less immunogenic vaccines (H120; H52 and D1466) may permit mutation (shift and drift). Although IB problems have decreased at the moment, it is necessary to keep the situation in the field under close surveillance. The findings mentioned above indicate the risk of the development of IB viruses which cannot be controlled with the available vaccines.

REFERENCES

- Alexander, D.J. and Chettle, N.J. 1977. Procedures for the haemagglutination inhibition tests for avian infectious bronchitis virus. *Avian Pathology*, 6, 9.
- Bijlenga, G. 1975. Het infectieuze bronchitis virus bij kuikens in Nederland aangetoond met behulp van ei-enting, dierexperimenteel onderzoek en serumneutralisatieproeven. *Tijdschrift voor Diergeneeskunde*, 81, 43.
- Davelaar, F.G., Noordzij, A. and Donk, J.A. van der, 1982. A study on the synthesis and secretion of immunoglobulins by the Harderian gland of the fowl after eye drop vaccination against infectious bronchitis at one-day-old. *Avian Pathology*, 11, 63.
- Davelaar, F.G., Kouwenhoven, B., Burger, A.G. and Lutticken, D. 1983. Signification des Serotypes variants de la brochite inf. aviaire aux Pays-Bas. *L'aviculteur*, 436, 5.
- Davelaar, F.G., Kouwenhoven, B. and Burger, A.G. 1984. Occurrence and significance of Infectious Bronchitis variant strains in egg and broiler production in the Netherlands. *The Veterinary Quarterly*, 6, 144.
- Jaspers, D., Hemmes, W. Weststrate, M.W. and Davelaar, F.G. 1984. The use of live and inactivated infectious bronchitis vaccines including Massachusetts and IB-variant serotypes in broiler-breeder and commercial layer flocks. *Proceedings XVII World's Poultry Congress, Helsinki*.
- Kouwenhoven, B., Davelaar, F.G., Burger, A.G. and Lutticken, D. 1983. Significance of variant IB serotypes in the Netherlands. *Australian Veterinary Poultry Association; the international union of immunological societies - proceedings no. 66*, 53.
- Lukert, P.D. 1975. Infectious bronchitis. In: *Isolation and identification of avian pathogens*. Hitchner, S.B., Domermuth, C.H., Purchase, H.G. and Williams, J.E. eds. pp 182 *Am. Assoc. Avian Pathologists*.

TABLE 14

Relationship of isolates with the serotypes D 274, D 1466, D 3128 and H 52

- Winterfield, R.W. and Fadly, M.A. 1975. Potential for polyvalent infectious bronchitis vaccines. American Journal of Veterinary Research, 36, 524.
- Wogernle, H. 1959. Diagnose der Infektiosen Bronchitis der Huhner mit Hilfe der prazipitations Reaktion im festen Agar Medium. Monatshefte fur Tierheilkunde, 11, 154.