(3)

Poultry

Gumboro Vaccine Nobilis® Strain 228E

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Summary

Since 1987 a highly virulent strain of the classical infectious bursal disease virus has been causing clinical disease problems in the field. This virus is giving high mortality in young pullets and also in broilers.
Even two or three times vaccination with the normal intermediate vaccines could not prevent the disease.
The problem is that the field virus can pass a higher level of maternal antibodies than the vaccine virus can.
So, before you can vaccinate a flock the field virus is already present.
Therefore this a little bit more invasive strain 228E was developed to be able to vaccinate birds with a quite high level of maternal antibodies.
With D78 you can vaccinate a bird with a VN titre of 2log 7 or lower.
With 228E you can vaccinate a bird with a VN titre of 2log 9 or lower.

Field trials with millions of birds are described in this article, proving that 228E is a very effective tool to prevent outbreaks of very virulent infectious bursal disease. Also in the Doorn trial it was proven that the vaccine had no negative influence on economic important parameters as growth per day, feed conversion, mortality or production index.

In the Netherlands where this vaccine was used on a big scale, the Gumboro outbreaks totally disappeared. Half a year after the last outbreak on a farm people go back to use D78.

1. Introduction

As Gumboro Vaccine Nobilis Strain 228E is fully registered in Holland since november 1991, it is important to give some more information about this very effective instrument to prevent field out breaks of very virulent classical Infectious Bursal Disease Virus.

Since 1987, a highly virulent strain of infectious bursal disease virus (IBDV), or Gumboro disease virus, has been causing clinical disease problems in the field.
The causal virus, which has been isolated in Western Europe, South Africa, Thailand and in the Middle East, is very virulent when compared to all previously isolated Gumboro viruses. In SPF chickens, infection with this virus results in mortality rates of 80 to 100%. These rates are extremely high when compared to the 40% mortality caused by the "standard" virulent Gumboro virus 52/70 (Faragher).

The first outbreaks generally occur in broilers at the end of the fattening period, after the fourth week. Subsequently, the disease appears earlier in successive flocks on the same farm due to endemic infection.
Mortality rates vary from 5 to 25%.
In replacement pullets, mortality rates of up to 60% are observed, suggesting a difference in susceptibility between layer and broiler chickens.
The course of the disease is around 6 days. In infected flocks, the clinical signs are not specific but include lethargy, ruffled feathers, watery diarrhoea due to increased water intake and lower feed consumption, and sudden death. Typical lesions include haemorrhagic and enlarged bursae, degenerative livers, haemorrhages in the thigh muscles and kidneys with swollen tubules.

Research conducted at The Poultry Health Institute in Doorn, The Netherlands, showed that this very pathogenic field virus could break through a higher level of maternal immunity than the vaccines which were on the market at that time. This was also proven in the field where even after several vaccinations in a flock outbreaks of Gumboro with high mortality occurred.

In contrast, in the laboratory SPF chickens proved to be protected against a challenge with a pathogenic Gumboro field isolate when they had been vaccinated 7 days before with a normal intermediate vaccine.
The problem is therefore that, in areas where this pathogenic Gumboro Virus is endemic, the birds get field infectionbefore the vaccine can overcome the maternal antibodies.
What was needed was a more invasive Gumboro vaccine : Gumboro Vaccine Nobilis Strain 228E.

2. The Gumboro Vaccine Nobilis Strain 228E

The original material used for the development of Gumboro Vaccine Nobilis Strain 228E was a field virus isolated from a non-vaccinated flock of broiler chickens.
At the time of isolation, the flock was suffering from a mild form of Gumboro disease demonstrated by histological, pathological and clinical investigations.
After isolation, the virus was attenuated by 62 consecutive passages in SPF chicken eggs.

3. Safety

In a trial in which two-weeks old SPF chickens were vaccinated with a normal dose of the Gumboro Vaccine Nobilis Strain 228E, it appeared that the virus multiplied very rapidly in the bursa.
Three days after vaccination, a large amount of virus could be detected in the bursa using the antigen capture Elisa test.

Of course, this causes depletion of lymphoid cells in the bursa, but a very quick recovery could be seen. This indicates a quick recovery of bursal function.

However, this vaccine is not meant for use in chickens without maternal antibodies. It is designed to protect chickens with moderate to high levels of maternal antibodies. In such cases it has very little influence on the bursa.

At the Animal Health Service in Boxtel, the Netherlands, a trial was done with maternally immune White Leghorn chickens.

Table 1: **Results of histological examination of the bursa taken at 4 days post vaccination.**

Vaccination age	vaccine	VN titre in ^{2}log at time of vacc.	Histology of the bursa RL[1]	Oedema	Lesions
5 wks	228E	7.5	2/5[2]	2/5	0/5
4 wks	228E	8.7	0/10	0/10	0/10
3 wks	228E	10.9	0/10	0/10	0/10

[1] RL = Reduced lymphocytes
[2] Number of chickens showing this phenomenon/number of chickens tested

From this trial it was concluded that when the titre at the moment of vaccination is higher, the influence on the bursa is smaller. This means that the vaccine can safely be used in chickens having moderate levels of maternal antibodies.

4. Laboratory trials

The following laboratory experiment was carried out in order to evaluate the capacity of Gumboro Vaccine Nobilis Strain 228E to give protection against Gumboro in WL chickens with maternally derived antibodies.
Three groups (1-3) of highly immune WL chickens were vaccinated with a widely used commercially available, intermediate type vaccine (vaccine A) at either 3 weeks, 4 weeks or 5 weeks of age.
Three other groups (5-7), hatched from the same parent stock, were vaccinated with Gumboro Vaccine Nobilis Strain 228E at the same ages.
Groups 1 to 3 were housed in a Fapp (= filtered air positive pressure) house in cages.
Group 4 were SPF birds not vaccinated, but housed in the same Fapp house, to check if the vaccine spreads to unvaccinated controls.
Groups 5 to 7 were housed in another Fapp house, also in cages.
Group 8 were SPF birds not vaccinated, but housed in the same Fapp house, to check if the 228E vaccine spreads to unvaccinated controls.
Groups 4 and 8 were four weeks old when the other groups were vaccinated.
Vaccination was done by individual oral instillation.

Table 2: **Results of vaccination WL chickens with a high level of maternally derived antibodies.**

Vaccine	Group	Age at vaccination	^{2}log VN titre at day of vaccination	AGP 2 wks p.v.[1]	4 wks p.v.
A	1	5 weeks	8.8	0/5[2]	0/5
A	2	4 weeks	9.2	0/10	0/10
A	3	3 weeks	11.1	0/10	0/10
-	4	—	<5.0	0/5	0/5
228E	5	5 weeks	7.5	5/5	5/5
228E	6	4 weeks	8.7	10/10	10/10
228E	7	3 weeks	10.9	6/10	6/10
-	8	—	<4.3	4/5	4/5

[1] Post-vaccination [2] Number positive from number tested

4.1 Conclusions

- Vaccine A did not result in seroconversion in 5 weeks-old WL chickens, while 228E resulted in seroconversion in 3 weeks old chickens.
- 228E vaccine spread easily to unvaccinated SPF chickens in cages, while vaccine A did not. This is a very important feature.
  In practice, there will always be a variation in titres of individual chickens on the day of vaccination. The quicker the vaccine spreads within a flock, the smaller the chance that the field virus will be able to break through.

Eight days after vaccination, groups 1 to 8 were challenged with a virulent Gumboro virus (Faragher IBD 52/70, 100 CID_{50} per animal). This challenge proved that the groups vaccinated with 228E had immunity against Gumboro, while the groups vaccinated with Vaccine A had no immunity, except in the case of group 3 which still had maternal immunity. Also, group 8 (the in-contact SPF birds with the 228E vaccinated groups) had good immunity, while group 4 (the in-contact SPF birds with the A vaccinated groups) had absolutely no immunity.

4.2 Conclusion:

- 228E Vaccine can break through higher level of maternal antibodies and spreads more within a flock than do the widely used "mild intermediate" vaccines (Vaccine A is a representative of this group).

5. Vaccination time

During the big field trial in the Netherlands, people at the Poultry Health Service in Doorn developed a formula to calculate at which day the birds can be vaccinated after having done an Elisa IBD (dilution 1:500) at day old.
Till now, only a formula for broilers has been developed.

In this trial 24 blood samples were taken per broiler house. When the chickens came from more parent flocks, then chickens from every parent flock should be sampled in a proportional way. The square root is taken from the individual Elisa titres and afterwards the average is determined.
This square root operation is done because the decline of the flock average during the time becomes then a straight line.

From the average 22,36 is subtracted (22,36 is the square root from 500, and this is the titre where the birds can be vaccinated).
The figure we have now has to be divided by 2,82 (this because the flock average from the square root treated titres goes down every day 2,82).
The figure we have now +1 gives the age in days when the flock can be vaccinated with Gumboro Vaccine Nobilis Strain 228E (this one has to be added because the flock is blood sampled at day 1 and not at day 0).

By using this formula we can predict the vaccination age with a reliability of 0,7 day.

Vaccination age in days is :

$$\frac{\text{Average from } \sqrt{\text{individual titres}} - 22{,}36}{2{,}82} + 1$$

6. Field trials

6.1 Broilers

Based on the good results obtained from the laboratory trials, a series of field trials was organized in order to evaluate the effectiveness of Gumboro Vaccine Nobilis Strain 228E under field conditions.
These trials were carried out on 5 broiler farms with a "Gumboro" history.
Vaccination was done via the drinking water.
The time of vaccination was determined by measuring the quantity of maternal antibodies against Gumboro at day one with an Elisa test and a VN test.

The earliest vaccination age was 8 days, the latest was 18 days.
The lowest ^{2}log VN titre at the time of vaccination was 7.4 while the highest was 9.9.
All flocks seroconverted after vaccination as determined in both in the AGP test and in the VN test.
None of the flocks suffered from Gumboro and the economic results were very good.
The mortality figures and the production indices of the 228E vaccinated flocks are given in Table 3 and compared with either the same data from a previous flock on the same farm (E-1) or with another house not vaccinated with 228E on the same farm (E-0) at the same time.

Table 4: **Results of vaccination on broiler farms with a "Gumboro history"**

Flock	Flocks 228E vaccinated %mort.	Prod. index	Flocks not 228E vaccinated Relation	%mort.	Prod. index
LH	5.6	197	(E-0)	8.6	173
HS	4.6	213	(E-1)	9.1	164
NH	3.1	215	(E-1)	6.0	195
MH	4.8	220	(E-1)	6.3	228
CS	4.8	199	(E-1)	7.3	200
Average	4.6	209		7.5	192

Production index = $\frac{\%\text{ birds alive x grams of growth per day}}{10\text{x feed conversion}}$

6.2 Layers

Similar experiments were carried out on pullet rearing farms.Here again, real "Gumboro problem" farms were selected. One farm had suffered from Gumboro in four consecutive flocks. The last flock had been vaccinated 11 times (every 3 days, starting at day 10). Despite this, an outbreak occurred at 7½ weeks with 20% mortality.
Rearing flocks were vaccinated twice with Gumboro Vaccine Nobilis Strain 228E, with an interval of 7 days.
This was done because the maternal antibody level in rearing flocks is less uniform than in broiler flocks.

The first vaccination with Gumboro Vaccine Nobilis Strain 228E was done at an age of between 17 and 26 days of age.
The ^{2}log VN titres on the day of vaccination varied from 7.6 to 10.5.
The flocks seroconverted after vaccination and none suffered from Gumboro.
Table 5 gives the mortality figures up to 17-18 weeks of age in the 228E vaccinated flocks compared with the same figures from previous flocks (not Gumboro Vaccine Nobilis Strain 228E vaccinated) on the same farm (E-x).

Table 5: **Results of vaccination on pullet rearing farms with a "Gumboro history"**

Flocks 228E vaccinated Flock	% mort.	Flocks not 228E vaccinated Relation	% mort.
1	3.7	E-1	30.2
		E-2	7.1
		E-3	4.8
2	1.4	E-1	20
		E-2	7
		E-3	7
		E-4	9

6.3 Conclusion:

- From these trials it can be seen that Gumboro Vaccine Nobilis Strain 228E is extremely helpful in solving Gumboro problems in the field. Also, no adverse reactions were seen at any time.

South Africa

6.4 Broilers

In South Africa, one integration conducted a large field trial utilizing Gumboro Vaccine Nobilis Strain 228E (see figure 1.). 2.77 Million day-old broiler chickens were involved, housed in 145 broiler houses.
Fifty percent of the chickens were vaccinated once with Gumboro Vaccine Nobilis Strain 228E, and fifty percent were vaccinated three times using a "normal" intermediate type Gumboro vaccine.
After vaccination with Gumboro Vaccine Nobilis Strain 228E at 14 days of age, no evidence of mortality or growth depression was seen. The difference in losses for the period 22-42 days was 2.6% less mortality in the broilers vaccinated with Gumboro Vaccine Nobilis Strain 228E.
In addition an advantage in growth rate was seen of 30 grams per chicken.
No evidence of immunosuppression was seen as measured by antibody development after normal Newcastle disease vaccination programmes.

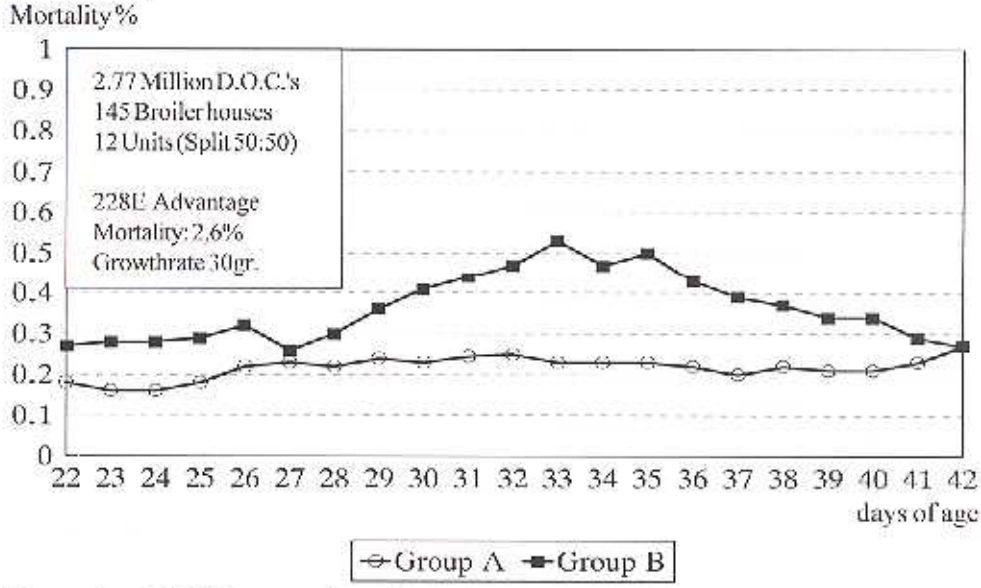
"Doorn", The Netherlands

6.5 Broilers, pullets and broiler breeders.

Because considerable Gumboro problems had been seen in the field in The Netherlands during 1990, the government, under pressure from the poultry industry, allowed the Poultry Health Institute in Doorn to prescribe Gumboro Vaccine Nobilis Strain 228E for use on Gumboro problem farms although it was not yet registered at that time. Under this permission, nearly 8 million doses of Gumboro Vaccine Nobilis Strain 228E were used between October 1990 and the end of March 1991.
Most of it was used in broilers, but a part also in pullets and broiler breeders.

Although all these 8 million doses were used on problem farms, (farms which had suffered from Gumboro in the previous flocks) only four small flocks (23,250 birds total) experienced outbreaks of Gumboro after using the vaccine. Total mortality from these 4 outbreaks was 624 birds (3%).

Figure 1: **Comparison of daily mortality after vaccination with different vaccines on a big broiler integration**



Group A: 228E (once vaccinated)
Group B: Intermediate Type (3 times vaccinated)

In three out of these four outbreaks, the disease started one or two days after vaccination and this suggests that, in fact, the time of vaccination was too late.
In this trial an investigation was also made to see if the stronger Gumboro vaccine had a negative influence on growth, feed conversion, production index or percentage of downgrading. No negative influence could be detected on these very important economic parameters.

7. Conclusions :

- Intermediate vaccines, such as the Intervet Gumboro Vaccine Nobilis D78 have been shown to be capable of efficiently preventing Gumboro outbreaks in commercial birds.
- In situations where infection pressure is high and very pathogenic Gumboro strains are present, breakthroughs can, however, occur. Gumboro Vaccine Nobilis Strain 228E is a more invasive vaccine which is able to penetrate maternal immunity at an earlier age.
- The vaccine has proven to be extremely efficient in solving serious Gumboro problems caused by very pathogenic strains.
- No negative side effects on economically important traits could be detected.

8. Vaccination

The best method of administration of Gumboro Vaccine Nobilis Strain 228E is via the drinking water.
The vaccine has been shown to be capable of breaking through a maternal antibody level of ^{2}log 9, expressed as virus neutralization titre.
This is an Elisa titre of approximately 500 (dilution 1:500). This does not mean that the vaccine absolutely does not work in the presence of higher antibody levels, but that the vaccination response takes longer and is not complete.
It is important that the parent stock has a uniform level of antibodies against IBD.
Experience has shown that, following the use of IBD emulsion, vaccinated flocks (progeny are "emulsion chickens") have a more uniform titre than that seen in flocks where no IBD emulsion has been used (progeny are "non-emulsion" chickens). It is therefore easier to determine the optimum time of vaccination for "emulsion" chickens than it is for "non emulsion" chickens.
For both groups it is best to determine the maternal antibody status at day-old and to calculate when the chickens can be vaccinated.
In broilers the titre goes down one log step every 4 days, and in layers this happens in 4.5 to 5 days.
In general, it is not wise to vaccinate very early, unless Gumboro breaks occurred very early, in the past.

Vaccination time:
The vaccination programme depends largely upon the local situation. As a guideline the following programme can be used :

Broilers :

	Vaccination age :
"Emulsion" chickens	day 14 - day 17
"Non emulsion" chickens	day 8 - day 12

Layers / breeders :

"Emulsion" chickens	day 21 + day 28
"Non emulsion" chickens	day 14 - day 21