

Elisa monitoring improves your

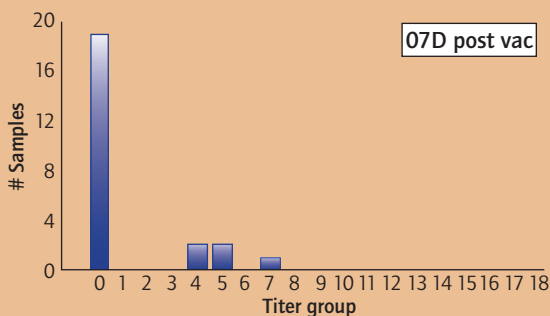
Vaccination is no guarantee that your birds do not become sick. Problems sometimes occur even when birds have been vaccinated against specific diseases. Has the failure something to do with the quality of the vaccine? Maybe, but more often, the problem is the result of poor vaccine handling and/or poor application. Elisa monitoring helps to improve the results and to detect the cause of failures.

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When vaccines are used from reliable sources, in general there should be no reason to blame these for poor results in disease control. The cause of the problem can often be found much nearer to the production facilities. Poor vaccine handling and incorrect vaccine application are more often to be blamed. A solid health management programme therefore is not complete without a checklist on which the effect of every handling is recorded. The use of Elisa serology has been widely accepted and proves to be a useful tool to monitor the immune response following vaccination.

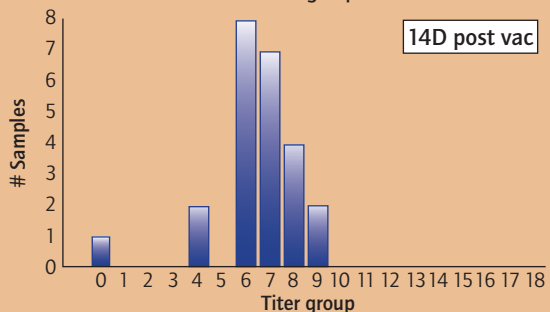
Case 1: Evolution of titers in IBD vaccinated broilers

Broilers were vaccinated at 14 days through drinking water with Intermediate Plus Vaccine. Titters were taken at 07D, 14D, 21D, and 28D post vaccination. Results show first 20 % seroconversion to take place at 07 D post vaccination, with 96%, and 100% seroconversion at 14D and 28D, respectively. Results over time show a typical pattern of decrease in % CV, coupled with rising mean titers. Titters at processing, 28 D post vaccination (42D of Age), show successful vaccination, as indicated by "Mean Titer" and "% CV", which are within range of expected results.



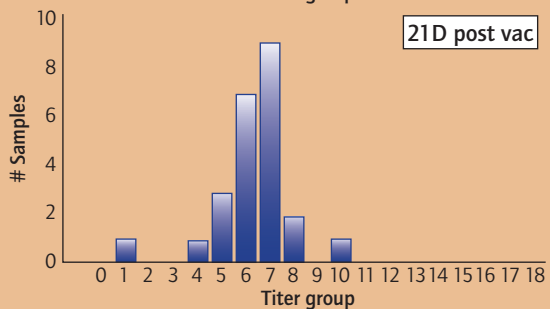
Assay: IBD
Bleeding date: 13-10-1998

Mean titer: 854
G.M.T.: 157
%CV: 197



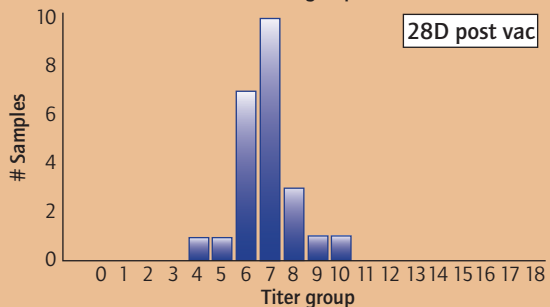
Assay: IBD
Bleeding date: 20-10-1998

Mean titer: 6291
G.M.T.: 5242
%CV: 44



Assay: IBD
Bleeding date: 27-10-1998

Mean titer: 5948
G.M.T.: 5200
%CV: 41
Target titer: 2500 - 8000
Target %CV: 30 - 45
Interpretation titer: OK
Interpretation CV: OK

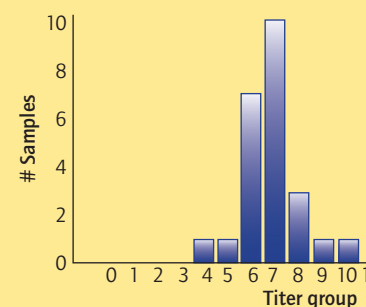
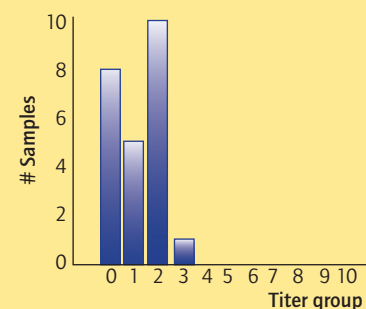
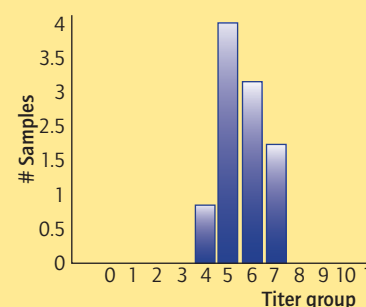


Assay: IBD
Bleeding date: 02-11-1998

Mean titer: 6801
G.M.T.: 6435
%CV: 33
Target titer: 2500 - 8000
Target %CV: 30 - 45
Interpretation titer: OK
Interpretation CV: OK

Case 2: How accurate

Samples were taken from a broiler flock at 01 D of age. Vaccination date prediction was applied with the "Deventer Method", predicting for a hot vaccine to vaccinate at 14 D of age (Optimal titer to vaccinate = 500, HalfLife MAB = 3.8 days, and Vaccination Cover = 75%). The grower wanted to know how good the estimated vaccination date was. He decided to take a



results

The main reasons for using Elisa for vaccination monitoring are the following:

1. You can evaluate the level of success of vaccination (Perform QC on vaccination)
2. You can diagnose vaccination failures
3. It allows you to take corrective action when vaccination failed
4. You can improve, optimise and maintain the efficiency of a vaccination programme
5. It justifies the investments in vaccination and the use of quality vaccines

When conducting serological monitoring of vaccinated flocks, one has to know basically

Is your vaccination date prediction?

sample at predicted age of vaccination and to take a sample at processing to determine the final level of success of vaccination.

Results showed that at the predicted age of vaccination (14 days), the actual mean flock titer of 400, nearly matched to Optimal titer to vaccinate (500). The accuracy of prediction and success of vaccination was further reflected in the high and uniform final titers (100% positive) at processing age (41D) of the flock.

Broilers 01D
Vaccination date prediction: (Deventer Log2 Method) 14 D of age

Assay:	IBD
Bleeding date:	22-09-1998
Mean titer:	4601
G.M.T.:	4337
%CV:	35
Target titer:	2000 - 8000
Target %CV:	30 - 45
Interpretation titer:	OK
Interpretation CV:	OK

13 14 15 16 17 18

Broilers 14D
Dr. Water vaccination with intermediate plus vaccine

Assay:	IBD
Bleeding date:	06-10-1998
Mean titer:	400
G.M.T.:	321
%CV:	59

13 14 15 16 17 18

Broilers 41D
Evaluation of vaccination: 100% positive titers High and uniform titers Successful vaccination!

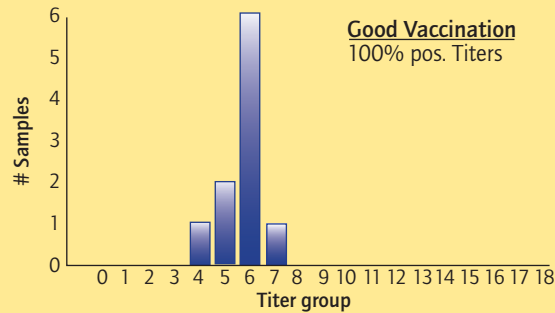
Assay:	IBD
Bleeding date:	02-11-1998
Mean titer:	6801
G.M.T.:	6435
%CV:	33
Target titer:	2500-8000
Target %CV:	30 - 45
Interpretation titer:	OK
Interpretation CV:	OK

13 14 15 16 17 18

Case 3: Control of IBD vaccination in broilers

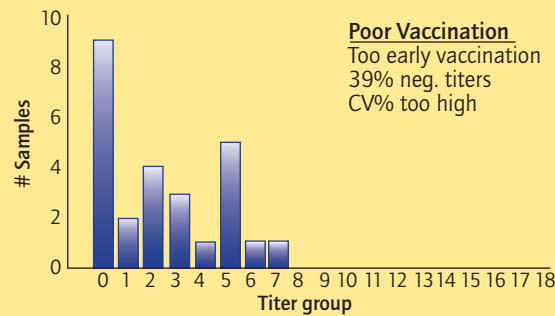
Broilers were vaccinated 1x with Intermediate IBD vaccine. To evaluate success of vaccination samples were taken at processing age and analysed. Shown below are 2 typical patterns one can find with monitoring: a successful vaccination

with high and uniform titers and a flock with 39% negatives. The failure of vaccination was caused by too early vaccination, resulting only in a partial take of the vaccination.



Assay: IBD
Bleeding date: 14-02-2000

Mean titer:	4593
G.M.T.:	4388
%CV:	30
Target titer:	2000 - 6500
Target %CV:	20 - 45
Interpretation titer:	OK
Interpretation CV:	OK



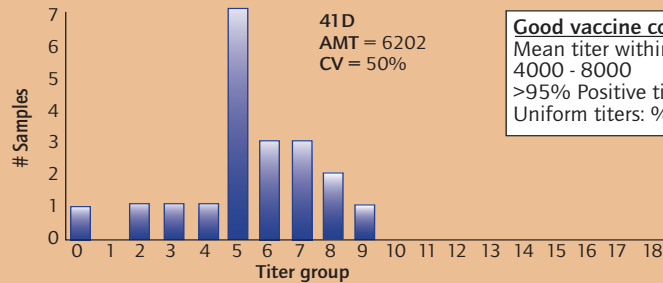
Assay: IBD
Bleeding date: 13-06-2001

Mean titer:	1523
G.M.T.:	476
%CV:	119
Target titer:	2000 - 6500
Target %CV:	20 - 45
Interpretation titer:	LOW
Interpretation CV:	HIGH

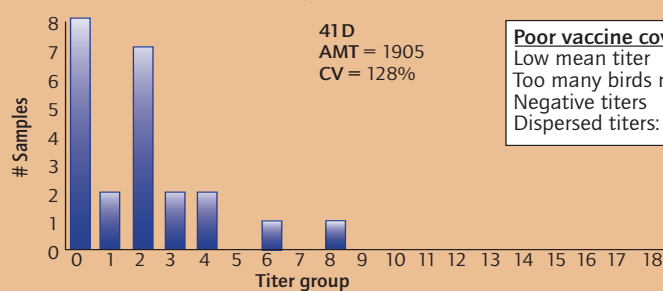
Case 4: Control of NDV vaccination technique

Broilers were vaccinated with a coarse spray at the hatchery. At 18 days broilers were revaccinated with a live vaccine with an atomist spray. Samples were taken at processing age to evaluate the success of vaccination. When evaluating results, one can easily recognise proper and improper

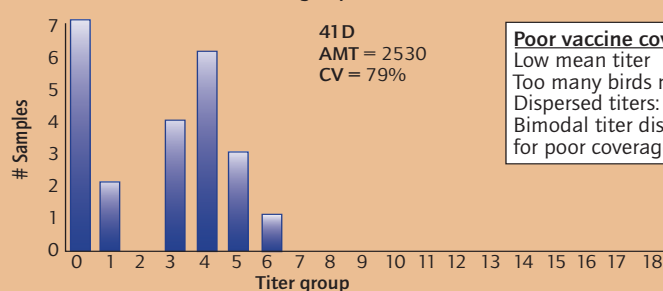
vaccination techniques, by looking at the pattern of ELISA histograms. Some typical patterns are shown that are associated with good application techniques and bad application techniques.



Good vaccine coverage
Mean titer within expected range = 4000 - 8000
>95% Positive titers
Uniform titers: %CV <60%



Poor vaccine coverage
Low mean titer
Too many birds missed >30%
Negative titers
Dispersed titers: %CV >60%



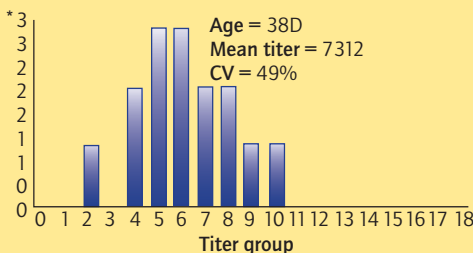
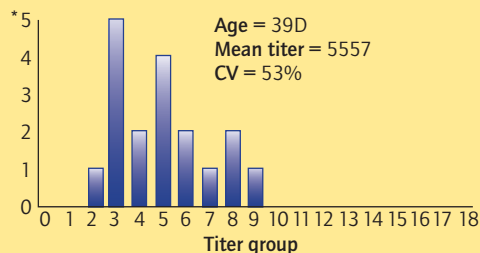
Poor vaccine coverage
Low mean titer
Too many birds missed
Dispersed titers: %CV >60%
Bimodal titer distribution typical for poor coverage

Case 5: ND atomist spray vaccinations and their effect

A serological survey at the processing plant of a large integrated company was conducted on broiler flocks from farmers and contractors. All broiler flocks tested were live vaccinated with ND (HB1) with a coarse spray in the hatchery. A second live ND vaccination was given with a ND LaSota at day 16, preferably with atomist spray. At time of processing, 24 serum samples were taken at random from a given broiler flock and the farm manager or contract farmer was interviewed about his vaccination procedure and disease history. From the serum samples a ND ELISA was performed to evaluate the success of vaccination.

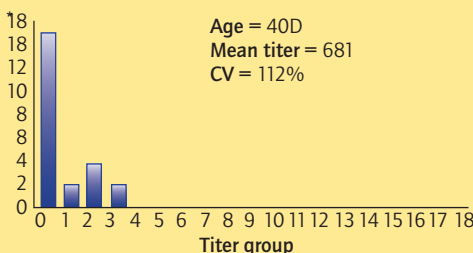
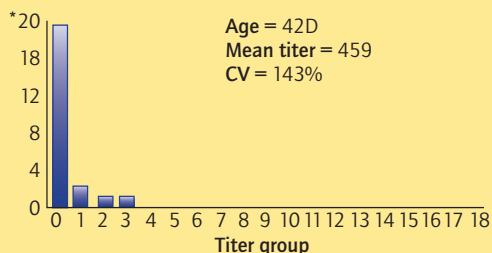
The study revealed that, one could recognise proper and improper vaccination techniques, by looking at the ELISA histograms. Shown below are some of the most frequent vaccination mistakes with the corresponding ELISA histogram results.

Good vaccination results: Correct spray application



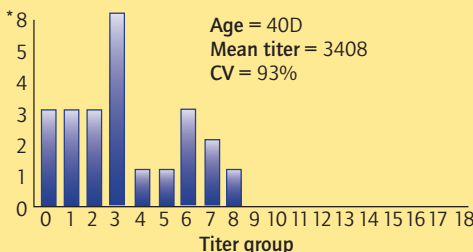
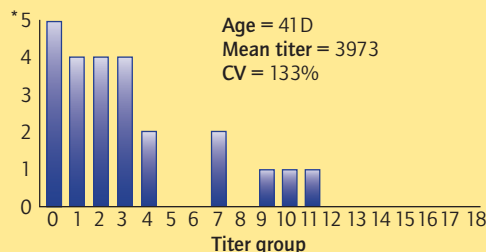
High and uniform titers, %CV <55%, 100% of the birds test positive

Poor vaccination results: Bad water quality



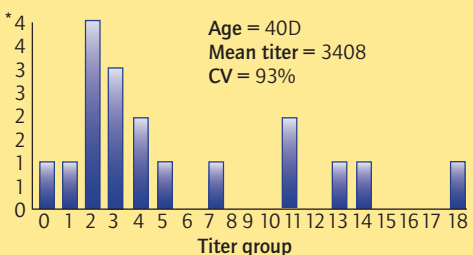
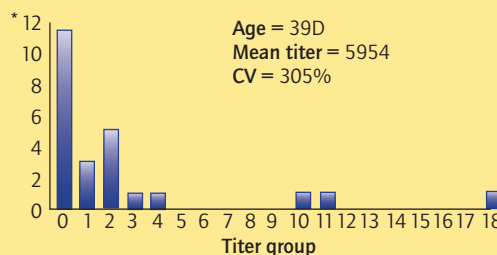
Mostly negative titers, very few positive birds

Poor vaccination results: Poor coverage



Sub-optimal mean titer, %CV, too many birds have been missed. Bi-modal titer distribution indication of poor vaccine coverage. Vaccination done in a hurry. Ventilation fans have not been turned off.

Poor vaccination results: Poor coverage, rolling vaccine infections

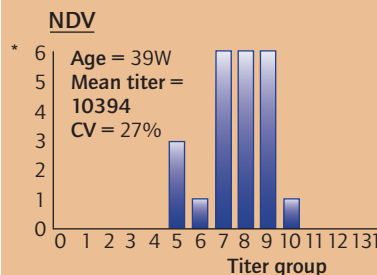
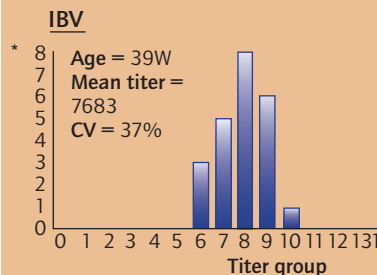
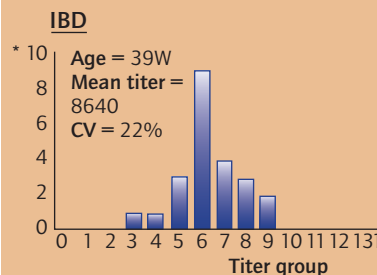


Good/high mean titers, very high % CV. Many birds with low titers, few birds with extremely high titers. ND-Spray vaccination performed in lack of time. Indications of "Rolling vaccine infections".

Case 6: Influenza

Broiler breeder flocks were vaccinated at 18 Weeks with inactivated ND+IB+IBD vaccine. ELISA results at 39W revealed some major differences between flocks. Throughout most of the year, the ELISA results were good, until during summer holiday, when the results began to show suddenly very poor and non-

Good vaccination Normal vaccination crew



two things beforehand:

- Which results to expect prior to testing (set standards for successful vaccination and interpret results by comparing obtained results with standards)
- What action to take if results are not according to expectation.

Interpretation of results

Interpretation of vaccination results is usually done by evaluating the three main key components of an immune response after vaccination, which are:

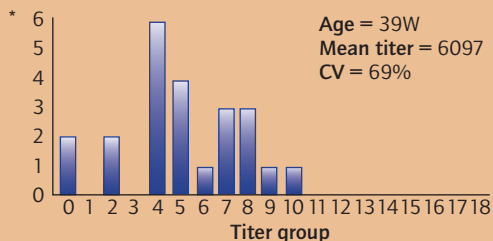
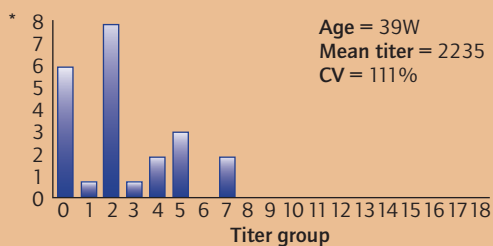
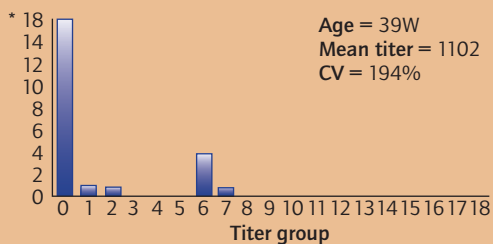
1. **Intensity** of response, as indicated by the Mean Titer. Do the birds develop sufficient titer levels that are in the expected range for the vaccine used? These expected titers following vaccination are of-

Vaccination crew on titer uniformity

uniform titers. Further investigation revealed that during the summer holiday the regular vaccination crew was replaced by a temporary vaccination crew, which explained the poor quality of vaccination during summer holidays. The flocks vaccinated by the regular vaccination crew had high and uniform ($CV < 40\%$) titers, whereas other flocks vaccinated by a temporary holiday crew, revealed very poor and non-uniform ($CV > 65\%$) titers

Poor vaccination

"Holiday replacement" crew



- ten called "Standard Titers" or "Baseline Titers" These Baseline titer values may vary according to type of bird, age, vaccine type, vaccination programme, and other factors. Therefore, one should make their own baselines for their own vaccination programmes and local conditions.
- Uniformity** of response, as indicated by the % CV. Is the vaccine actually received by all the birds or not? Is the %CV within the required range or is there room for improvement? The general guidelines for %CV following vaccination are:

% CV	Uniformity
Less than 40%	Excellent
40-60%	Good
Higher than 60%	Need to improve

Note: With live priming of breeders, complete seroconversion is more important than %CV. Check if 100% of the birds test positive

- Persistency** of response, as indicated by Mean Titer response over time. Do titers persist long enough over time, or is another vaccination needed to boost titers above minimum protective levels? Indicators of successful vaccination are generally high, uniform and lasting titers that are within the expected range for the type of vaccine. Also these titers should be 100% positive. Indicators of poor vaccination efficiency are generally the opposite: i.e., Titers lower than expected, non-uniform, and non-persistent. These "below the baseline" titers are usually associated with moderate to high % of negatives. For some vaccinations, such as AE and CAV, % seroconversion is the only meaningful indicator of success. For instance for AE, if $> 60\%$ test positive after vaccination, re-vaccination is no longer required. To illustrate the above points on interpretation and most practical use, the ELISA as a "Quality Control" method to evaluate and optimise your vaccinations, a number of case histories and field data are shown.

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Conclusion

From the case histories it can be concluded that poor administration and/or vaccination techniques are the most common cause of vaccine failure in poultry. Results have frequently demonstrated that ELISA monitoring is useful for finding out if a vaccine has been correctly applied or not. If results are poor, it allows you to re-evaluate your vaccination procedures to find out what went wrong so you can take corrective actions. This way regular vaccination monitoring should improve the effectiveness of vaccine application and in turn improve disease control and economic performance of poultry flocks. h

Case 7: ND drinking water application vs. spray application

A grower wanted to investigate what the most effective way was to apply the 2nd live ND vaccination to broilers. He set up an experiment with 4 broiler flocks. All the 4 flocks were vaccinated at hatchery with live ND vaccine at half dose. At 14 days 2 flocks were vaccinated with live ND vaccine through drinking water and the other 2 flocks were vaccinated with the same live ND vaccine through coarse spray. The results showed (see Fig. 1), that

the coarse spray application produced higher mean titers than the drinking water treatment. Further analysis, by looking at % positive birds also revealed that Coarse Spray has the tendency to earlier seroconversion in between 7 and 21 days post vaccination (see Fig. 2). These findings convinced the grower to use coarse spray applications for future live ND vaccinations, instead of drinking water applications.

Figure 1. NDV: Results 4 broilers flocks vaccinated with Clone 30 at 01D & 14D drinking water vs coarse spray application

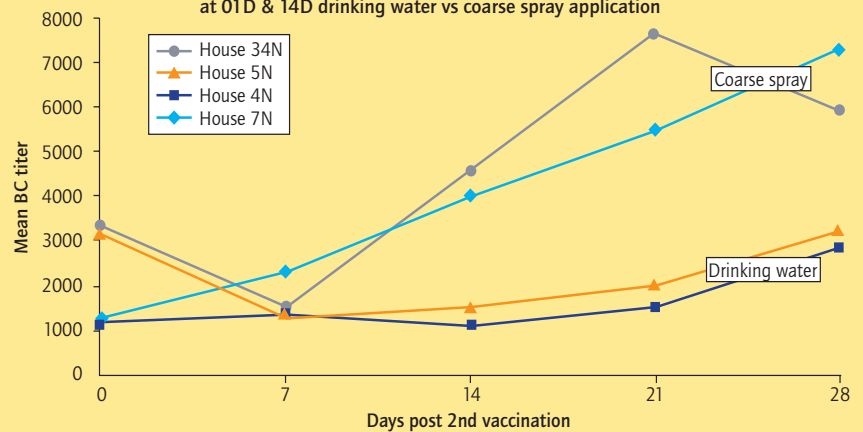


Figure 2. NDV: Results 4 broilers flocks vaccinated with Clone 30 at 01D & 14D drinking water vs coarse spray application

