Efficiency of Commercial Air Filters Against Marek’s Disease Virus

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In a series of 10 replicate trials with susceptible chickens as indicators of the presence of virus in the air, filter B1 (Dri Pak, American Air Filter Co.), which has a dust spot rating of 93 to 97%, and filter C (Astrocel), with a diocyl phthalate aerosol rating of over 99%, completely removed Marek’s disease virus (MDV) from highly contaminated air. However, air passed through filter B2 (Duracel), with a dust spot rating of 34 to 45%, remained infectious. Thus, an air filter with a dust spot rating of over 93% will effectively remove MDV from the air. The smallest infectious particles in the air are estimated to be greater than 0.3 μm in diameter.

Marek’s disease (MD) is a widespread, readily transmissible lymphoproliferative disease caused by a cell-associated herpesvirus (MDV; reference 4). Infection is present in most chickens and may persist for long and indefinite periods (12). The natural source of infectious virus appears primarily to be the feather follicle epithelium of infected chickens (2, 3), a site where virions develop to maturity, become enveloped, and are infectious in the absence of cells. The infectious virus probably exists in aggregates or in association with larger particles, rather than as individual virions. Poultry house dust and fine particles found under birds in cages have been found to be highly infectious (1, 8, 9).

Air surrounding infected chickens is also infectious as indicated by the high efficiency of airborne transmission (5, 10). Exposing susceptible chickens to infectious air for less than 30 min resulted in a high rate of transmission (J. H. Chen and R. L. Witter, unpublished data). This infectivity has presumably been removed by passage through filters with efficiency ratings of 90 to 99% for particles 1 to 10 μm in size (6, 11). However, in these tests there were no positive controls, i.e., infection without filters; thus, the function of the filters for retaining infectious particles could only be presumed.

Air filtration to remove infectious disease agents is a potential means of disease control under practical conditions, and interest in this procedure has been demonstrated by a number of poultry growers. Whether the use of such filters is practical under commercial conditions depends not only on their ability to remove disease agents but also on the operational cost per unit volume of filtered air which may vary between filters of different types and efficiencies. Only one type of commercial high-efficiency air filter was found to retain Newcastle disease virus (7). This study demonstrates the efficacy of commercial filters of various types in removing infectious MDV particles from the air.

MATERIALS AND METHODS

Source of MDV-contaminated air and design of equipment. The MDV-contaminated air was drawn under negative pressure (about 12 cm of water negative to the atmosphere) from about 55 Honsfall-Bauer isolators containing chickens or turkeys (Fig. 1). These birds were under various experiments but invariably included some groups of chickens infected with MDV. Room air was drawn through three layers of filter media into cage 1 (11). The contaminated air on the positive pressure side of the air turbine was divided among the cages (cages 2 and 3) which served as positive controls and the test filters in positions A, B, and C. After passing through the filters a portion of the air went through cages 4 and 5 used for bioassay and the remainder was exhausted from the building.

Filters. The commercial filters (American Air Filter Co., Louisville, Ky.) used were of four types. Each had a face dimension of 61 by 61 cm but varied in length. Filter A (Renu-Kleen) was 6.3 cm in length and had an air capacity of 566 liters per sec (LPS) at 0.5-cm water gauge pressure (WG). The filter efficiency was 78% by the weight test. The flow rate of air passing through the filters during the test
period was about 236 LPS. Filter B₁ (Dri Pak 100 series filter) was 45.4 cm in length and had an air capacity of 472 LPS at 1.3 cm WG with a working range of 1.3 to 2.5 cm. The filter efficiency was rated at 80% to 85% by the dioctyl phthalate aerosol (DOP) test with a particle size averaging 0.3 μm as determined by light scattering, 93 to 97% by the dust spot (National Bureau of Standards) test, and 99.6% by the weight test (American Filter Institute). Filter B₂ (Duracel filter) was 30.4 cm in length and had an air capacity of 944 LPS at 0.5 cm WG with a working pressure range of 1.3 to 2.5 cm. The filter efficiency was rated at 45% by the dust spot test and 91% by the weight test. Filter C (Astrocel filter) was 29.2 cm in length, had an air capacity of 542 LPS at 2.5 cm WG with a working pressure range of 2.5 to 5.1 cm WG, and a filter efficiency of 99.97% by the DOP test.

**Bioassay.** In general, assays of airborne MDV were conducted by passing the air through Horsfall-Bauer isolators containing 15 X 7 cross chicks which are highly susceptible to MD. Specifically, the test isolators were hooked up to receive filtered or nonfiltered air as shown in Fig. 1. About 24 hr after initiation of airflow, the 1-day-old chicks were banded and placed in the respective isolator cages. The test air was passed under pressure through cages 2 through 5 for the first 14 days. The air that passed through these cages was regulated by a gate valve located at the air entrance of each isolator, and the volume was estimated to be about 0.5 LPS. After the 14th day, each of these cages was attached to the negative pressure system and filtered room air was used in the regular manner (11). Each test on filtered or nonfiltered air was replicated, and, in addition, an additional lot of chicks was placed in a Horsfall-Bauer isolator (cage 1) which was supplied with filtered room air under negative pressure to serve as a negative chicken control. Chicken responses to 8 weeks were evaluated by both pathological and serological criteria as described earlier (11).

**Experiments.** For all trials, filter A was used as a prefilter to remove the larger dust particles. This prolonged the useful life of the higher efficiency filters located downstream. It was necessary to replace filter A at about 48-hr intervals.

The purpose of trial 1 was to test filter C which had the greatest efficiency. In practice, a medium-efficiency filter, such as filter B₁, is often employed as a prefilter to a very high-efficiency filter. Thus, for the first trial, filter B₁ was interposed between filters A and C. For trials 2, 3, and 4, filter C was removed and filter B₁ used in the first trials was left in place. For trials 5 and 6, filter B₁ was replaced with filter B₂. Trials 7 and 8 were conducted with a new filter B₁ with extra precautions against air by-passing the filter media. For trials 9 and 10, a new filter B₁ was tested with the same filter for both trials. At each installation of new filters, precautions were taken to disinfect the filter chamber and air tube connection.

**RESULTS**

None of the birds in either replicate group which received air that had passed through filter B₁ and C showed evidence of infection since they lacked antibody and lesions at 8 weeks (Table 1). In contrast, the birds that received unfiltered air all had MDV antibody and 7 of 16 had gross lesions.

Two series of tests were conducted with filter B₁. In the first series, trials 2, 3, and 4, the filter was the one used in the series with the filter C in trial 1. All six assay groups which received the air that had passed through this filter B₁ remained negative for MDV antibody and lesions. In contrast, all groups that received the unfiltered air became infected. A second filter B₁ was tested in trials 9 and 10.
TABLE 1. Efficiency of various filters for removing airborne MD infection

| Expt | Filter | Per cent efficiency | Responses of chickens to | Air filtered | Air not filtered | Unexposed control |
|------|--------|---------------------|--------------------------|-------------|-----------------|------------------|
|      |        |                     | Replicates               | Antibody    | Gross lesion    | Antibody         | Gross lesion    |
|      |        | DOP* | Dust spot* | API wt* |                | 0/7* | 0/7* | 7/7 | 2/8 | 0/8 | 0/8 |
| 1    | C      | >99 |           |        | 1              | 0/8 | 0/8 | 7/7 | 5/8 | 0/8 | 0/8 |
| 2    | B<sub>1</sub> | 80-85 | 93-97 | >99 | 1 | 0/8 | 0/8 | 8/8 | 5/8 | 0/8 | 0/8 |
| 3    | B<sub>1</sub> | 80-85 | 93-97 | >99 | 1 | 0/6 | 0/6 | 6/6 | 0/6 | 0/8 | 0/8 |
| 4    | B<sub>1</sub> | 80-85 | 93-97 | >99 | 1 | 0/7 | 0/7 | 7/7 | 2/7 | 0/7 | 0/7 |
| 5    | B<sub>2</sub> | 34-45 | 91 |       | 1 | 0/8 | 0/8 | 7/7 | 3/8 | 0/8 | 0/8 |
| 6    | B<sub>2</sub> | 34-45 | 91 |       | 1 | 0/8 | 0/8 | 7/7 | 3/8 | 0/8 | 0/8 |
| 7    | B<sub>2</sub> | 34-45 | 91 |       | 1 | 0/8 | 0/8 | 7/7 | 3/8 | 0/8 | 0/8 |
| 8    | B<sub>2</sub> | 34-45 | 91 |       | 1 | 0/8 | 0/8 | 7/7 | 3/8 | 0/8 | 0/8 |
| 9    | B<sub>1</sub> | 80-85 | 93-97 | >99 | 1 | 0/8 | 0/8 | 6/6 | 4/8 | 0/8 | 0/8 |
| 10   | B<sub>1</sub> | 80-85 | 93-97 | >99 | 1 | 0/8 | 0/8 | 8/8 | 5/8 | 0/7 | 0/8 |

* Filter C (Astrocel), B<sub>1</sub> (Dri Pak series 100), and B<sub>2</sub> (Duracel) were purchased from the American Air Filter Co., Louisville, Ky.
  * Diocyl phthalate aerosol, average of 0.3 μm as determined by light scattering.
  * National Bureau of Standards test with atmospheric dust of about 1 μm.
  * American Filter Institute "weight efficiency" test.
  * Number of birds positive for precipitating antibodies or gross MD lesions/total birds tested or examined.
  * Six of eight birds drowned at 42 days of age and three had gross MD lesions.
  * Killed at 6 weeks.

and similar results were obtained; i.e., none of the birds that received the filtered air became infected.

Filter B<sub>2</sub> was tested in two assay series. In the first series (trials 5 and 6), infection was obtained in the chickens of only one replicate of each trial that had received the filtered air. The other replicate of each trial remained negative for both antibody and lesions. The reason for the inconsistency was not apparent, and examination of the air duct on the downstream side of the filter on the 61st day revealed gross evidence of isolator dust. An air leak between the filter frame and the air duct was suspected but not proven.

In the second series of trials with another filter B<sub>2</sub>, extra precautions were used to provide an airtight seal between the filter and air duct. An examination of the air duct on the 31st day of the experiment revealed no evidence of dust. Test results (trials 7 and 8) show that replicate assays were consistent and confirmed the first series in that the filtered air caused infection in all assay chickens.

**DISCUSSION**

In the 10 trials consisting of 20 replicates, the infectivity of the test air was uniformly high as indicated by MDV antibody in 132 of 134 birds receiving nonfiltered air. Furthermore, there were gross lesions in birds among 19 of 20 positive control lots. There was some inconsistency between replicates in one series of trials with filter B<sub>1</sub> which cannot be explained. The remaining eight pairs of tests and the two series of tests with filter B<sub>2</sub> gave consistent results.

Filter B<sub>1</sub> with a dust spot rating of 93 to 97% removed all MDV from infected air, but filter B<sub>2</sub> with a dust spot rating of 34 to 45% did not remove the MDV under the conditions of these tests. However, it is possible that under other conditions results may differ. Two important variables to be considered in other tests are the volume of air passing through the filters and the dust "loading" of the filters. In these trials, the volume of air remained constant and was estimated to be 236 LPS which
is about 50% of the capacity of filters C and B1 and about 25% of the capacity of filter B2.

Obtaining an estimate of the "loading" of the filters is more difficult because it is largely determined by the air velocity, filtration time, amount of dust, and the size of the dust particles in the air. Although "loading" increases the efficiency of the filter, a breakdown in the media matrix may occur if air pressure on loaded filters increases above the working pressure. This could result in openings permitting the passage of larger particles. Increase in pressure drop across filters was for all trials minimal. Hence, the trials were conducted with filters having a minimum of "loading"; thus the working pressure was of insufficient magnitude to provide a severe stress on filter media. Also, in all trials, except trials 3 and 4, the filters were not previously used or were in use only 2 weeks and the filtration trials were of only 2 weeks duration. In trials 3 and 4, the filters employed had been in use 10 and 12 weeks, respectively. Commercial filters are available with filtration efficiencies intermediate between those of filters B1 and B2. It is possible that filters less efficient than filter B1 would completely eliminate MDV from contaminated air. These were not examined because they were not significantly less costly than filter B1.

Since filters having an 80 to 85% efficiency for particles of about 0.3 μm (filter B1) completely retain the MD infectious particles and filters having an efficiency of 34 to 45% for particles of about 1 μm (filter B2) do not retain the virus, all the infectious particles in the air are probably greater than 1 μm in diameter. Intact cells are much larger than 1 μm; however, cell parts, such as cytoplasmic inclusion bodies, fall within this range and are candidates for the smallest infectious particles in the air.

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