AIR HYGIENE IN TUBERCULOSIS: QUANTITATIVE STUDIES OF
INFECTIVITY AND CONTROL IN A PILOT WARD

A Cooperative Study Between the Veterans Administration, The Johns Hopkins
University School of Hygiene and Public Health, and the Maryland
Tuberculosis Association

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INTRODUCTION

From both clinical and laboratory studies has come evidence which converges
on the concept that pulmonary tuberculosis may be primarily air-borne. In
"open" cases, vast numbers of tubercle bacilli are harbored in the sputum;
vviolent expiratory processes such as coughing and sneezing atomize myriads of
tiny droplets into the air; nuclei remaining after instantaneous evaporation of
these droplets stay air-borne until breathed or vented; infective droplet nuclei
when breathed by animals are implanted on susceptible lung tissue where they
induce tubercles. If the chain of events suggested by these findings is substan-
tially correct, then the possibility of breaking the chain by sanitary control of
contaminated indoor air deserves consideration. In the light of the outstanding
success of sanitary control of water-borne and insect-borne parasites, air hygiene
offers a major challenge to public health.

Dr. John B. Barnwell, Director of Research and Education for the Veterans Administration,
is the man who initiated the study of air hygiene in tuberculosis and nurtured this scientific
baby to its present adolescent stage. Dr. Barnwell's interest, in turn, was largely the result
of Mr. William F. Wells' impressive laboratory studies at Harvard and the University of
Pennsylvania (1). These studies clearly indicated the need for a full-scale test in a pilot
ward. A suitable place for such a test was finally found at the Veterans Administration
Hospital in Baltimore, and Mr. Wells and his assistant, Mrs. Cretyl C. Mills, moved their
base of operations there. Through the extraordinary generosity of the University of Penn-
sylvania they brought with them Mr. Wells' calibrated animal exposure chamber and other
pieces of apparatus which have been of inestimable value in the present studies. Dr. Riley
became associated with the project because of his early interest in Mr. Wells' work in the
1930's and because of his connection with The Johns Hopkins University School of Hygiene
and Public Health. The Maryland Tuberculosis Association joined in by contributing funds
and encouragement at a crucial moment. Dr. Ross L. McLean, Director of Professional
Services at the Veterans Hospital, coordinates the laboratory and clinical aspects of the
project and provides essential liaison between the line and the staff within the VA. Dr.
Walen'ty Nyka, pathologist of the hospital, provides expert advice in his field and an
impartial check on estimates of the number of tubercles in animal lungs. Dr. Bruce Armstrong
gave invaluable support when the Baltimore project was in its infancy.

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Schenectady, New York, November 9, 1956.
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St., Baltimore 5, Maryland.
The present report deals with some of the studies which have been performed in the pilot ward to date. These preliminary steps include: control of ventilation; control of hazards from escaping bacteria; control of infection and disinfection within the ward; comparison of the specific infectivity of different strains of tubercle bacilli; design, construction, and operation of a special animal exposure chamber; and finally, calibration of the pilot ward. Each step has been essential in preparing for the major objective, which is to determine whether animals can be infected by breathing the air from the pilot ward when it is occupied by human patients with open tuberculosis, and whether such air-borne infection, if achieved, can be eliminated by means of air hygiene.

Observations

Figure 1 is a schematic picture of the pilot ward and its ventilating system. Six single rooms in a row open onto an inner corridor which in turn opens onto the main corridor of one of the regular hospital wards. Standard ultraviolet light fixtures which irradiate the air above head level are installed in each room. The pilot ward is on the top floor of the hospital and its own private air-conditioning system is located in a penthouse immediately overhead. As now arranged, exhaust air on its way to being vented outdoors passes through a large animal exposure chamber. Other modifications which were necessary will be described below.

Fig. 1. Schematic diagram of pilot ward and animal exposure chamber.
Figure 2 is a photograph taken from the inner corridor. The ultraviolet light fixtures over each door may be seen. The main hospital corridor is to the left. In figure 3 is shown the interior of one of the rooms with the ultraviolet fixture for irradiating the upper air.

Control of Ventilation

Control of ventilation was essential because the infectivity of ward air depends upon the amount of dilution of infective particles with outside air. The first step in preparing the pilot ward for the experiments was to reduce the intake of fresh air both by closing off the intake grill and by changing from an open circuit to a recirculation system. Determination of the total amount of fresh air entering the system posed a difficult technical problem because air entered through innumerable small leaks, particularly at junctions in the ductwork and around doors and windows. Furthermore, the pressure in part of the system was slightly above atmospheric and in other parts slightly below atmospheric. Mr. A. B. Hubbard of the Air Conditioning Division of the General Electric Company made a preliminary investigation of this situation, and estimates of total fresh air intake were then made by Mr. Carl W. Coblenz of the National Bureau of Standards, using a helium-dilution method. Without the help of these engineers, quantitative studies of infection and disinfection in the pilot ward would not have been possible.

With the helium-dilution tests used to monitor progress, control of ventilation

Fig. 2. Photograph of inner corridor of pilot ward.
was finally accomplished by a combination of direct and indirect approaches. Plastic curtains were stretched over the windows and other leaks were plugged as well as possible with Scotch® cellophane tape, cardboard, and plastic sheets. Then the fan in the ventilating system was slowed in order to lower the pressure within the system and thus reduce the amount of air passing through any remaining cracks. This changed the aerodynamics of the system, but the ventilation characteristics now satisfied the requirements of the program. Entering fresh air amounted to approximately 213 cu. ft. per minute, or roughly 40 cu. ft. per minute per patient. This value exceeds good public school ventilation standards, yet causes a dilution of contamination which is low enough, it is believed, to permit measurement of the infectivity of "open" cases of pulmonary tuberculosis. That this belief is more than a pious hope will become apparent after quantitative studies dealing with artificial contamination of the ward air are described.

Control of Hazards from Escaping Bacteria

Control of hazards from escaping bacteria depends partly upon minimizing the outward movement of air through leaks in the system and partly upon posi-
tive means of destroying organisms before they can enter the rest of the hospital. After numerous modifications, the ventilating system was so balanced that virtually all air left the system through the exhaust duct and very little through leaks in the system. This aerodynamic control reduced the likelihood of organisms escaping into the rest of the hospital, but did not provide a guarantee that occasional organisms might not escape from parts of the system where the pressure exceeded atmospheric.

The final guarantee was provided by a bactericidal barrier which enveloped the entire pilot ward and its air-conditioning system. Fortunately, the section of duct where the pressure was highest, hence the possibility of outward leakage greatest, was in the penthouse. The air surrounding this ductwork could be irradiated directly with ultraviolet light. Naked bactericidal burners attached to the high ceiling flooded the whole space with germicidal radiation which, it is believed, no parasite could survive for an instant. The inner corridor separating the ward from the hospital was converted into a bactericidal air lock by light curtains hung over the door to the rooms and to the outer corridor. The space between the ward ceiling and the penthouse floor, which carries the distributing ducts, was also heavily irradiated. No air escaping from these ducts could reach the hospital without passing through a deadly barrage of germicidal radiation. Indeed, the ceiling blocks at several points in the main corridor around the ward were replaced with grids through which ultraviolet light could stream downward, tending to convert these corridors from channels conveying air-borne germs into air purification chambers.

The bactericidal tightness of this all-embracing barrier was checked repeatedly by atomizing high concentrations of E. coli into the air at the conditioner and sampling wherever it seemed remotely possible that escaping organisms might be picked up. The absence of E. coli from samples of air elsewhere in the hospital, when thousands per sample were collected from pilot ward air, satisfied responsible authorities as to the tightness of control of the hazard of escaping bacteria.

Control of Infection and Disinfection Within the Ward

In the first tests, a broth culture of E. coli was atomized into the air-conditioning system and, after allowing time for equilibrium conditions to be reached, organisms in the exhaust air were sampled with the Wells air centrifuge. Tests were made with and without the ultraviolet lights on in the rooms. Only 2.5 per cent as many test organisms were recovered when the ultraviolet lights were on as when they were off. These findings immediately demonstrated the over-all efficiency of ultraviolet disinfection within the ward. However, additional tests in which samples were taken within individual rooms gave inconsistent results which were best explained as due to inadequate mixing of the air in the upper and lower parts of the rooms. The importance of circulation of air within each room, carrying infective particles quickly from the lower unirradiated to the upper irradiated zone, was brought strongly to attention by these tests. During subsequent animal experiments fans were placed in each room to circulate the air. At present this internal circulation is accomplished by a modification of the air-conditioning system.
Bovine tubercle bacilli were first atomized into the air-conditioning system of the pilot ward in March, 1955. Twelve rabbits were placed in one of the rooms, all the ultraviolet lights were turned on, the atomizer flask was filled with culture filtrate, and the atomizer was turned on. The rabbits breathed the air with the ultraviolet lights on for two hours. Then the atomizer was turned off and 6 animals were removed. All the ultraviolet lights in the rooms were then turned off, the atomizer was restarted, and the 6 remaining rabbits breathed the infected air for three hours. Again the atomizer was turned off and all the lights were turned on for purposes of decontamination.

Eighteen days after exposure, one of the second group of animals died, apparently from an intercurrent infection. A few minute specks on the lungs looked suspiciously like tubercles. The first one examined contained acid-fast organisms, and it was thus known that the animals had been successfully infected. The remaining rabbits from this group were sacrificed on various occasions, and the numbers of macroscopic tubercles in the lungs may be seen in table 1. An average of seven tubercles was present in each of the 5 rabbits in which accurate counts could be made. None of the rabbits breathing irradiated air showed any sign of infection. Five were sacrificed and showed no gross evidence of tubercles. The lungs of 3 animals were submitted to the Armed Forces Institute of Pathology for examination, and no tubercles or acid-fast organisms were found. A guinea pig inoculated with the ground tissue from another rabbit has failed to develop tuberculous infection. The final rabbit is still alive and well more than a year and a half after exposure. Thus, disinfection as well as infection has been demonstrated in the pilot ward.

**Specific Infectivity of Different Strains of Tubercle Bacilli**

The next phase consisted of standardizing the procedure in order to perform more accurate quantitative studies. The first step involved recovery of a strain of bovine tubercle bacilli of known infectivity. On the theory that the most highly infective organisms would be those which had actually produced tubercles in the rabbits, several cultures were recovered from resected tubercles. Five of these cultures were atomized into the calibrated inhalation apparatus and their infectivity for rabbits was compared. In each case organisms in the air which the rabbits breathed were collected in buffer solution in the air centrifuge, aliquots of the buffer solution were planted on solid media, and the colonies which subsequently developed were counted. Each colony represented a viable particle in the air which the rabbits had breathed. These counts were compared with the

<table>
<thead>
<tr>
<th>Ultraviolet Lights in Rooms</th>
<th>Tubercles per Rabbit*</th>
</tr>
</thead>
<tbody>
<tr>
<td>On</td>
<td>0</td>
</tr>
<tr>
<td>Off</td>
<td>2+</td>
</tr>
</tbody>
</table>

* All 12 rabbits exposed in Room 2.

[ ] Indicates interference with accurate count by intercurrent infection.
number of tubercles developing in the rabbits' lungs. In table 2 the average number of viable particles breathed, as determined by the cultural method, is compared with the average number of tubercles counted in the lungs. The first culture was called Ravenel VA because of the high ratio of tubercles to colonies. The apparent excess of tubercles over colonies is attributed to technical inaccu-

racies. Within the limits of error of the methods, each viable nucleus which was
inhaled produced a tubercle in the lungs. Ravenel VA is now used as the standard strain and, when grown and prepared according to standard procedures, can be used for quantitative studies.

When patients occupy the pilot ward, they will produce human strains of tubercle bacilli and not Ravenel VA. Accordingly, it was important to compare the behavior of human strains with the standard strain. Because of the limited number of untreated "open" cases among patients entering the hospital, the writers were particularly interested in determining whether patients producing drug-resistant organisms would be suitable test subjects. This decision hinged primarily on the infectivity of the patient's organisms as compared with Ravenel VA.

To date one isoniazid-resistant strain has been tested, and the results have yielded two major surprises of considerable importance. Culture filtrate containing a known concentration of organisms produced, when atomized, many times more than the expected number of viable nuclei, raising the possibility that this strain was more resistant than Ravenel VA to the trauma involved in changing from the aqueous to the air-borne state. In comparison with Ravenel VA the human strain produced about four times as many tubercles in guinea pigs. This important experiment is being repeated.

The second surprise related to the progress of the disease in the infected ani-

mals. There was suggestive evidence, based on the appearance of the lungs of the one guinea pig that was permitted to live for three months, that this drug-resistant human strain might be relatively avirulent. This finding is mentioned only as an indication that the technique of air-borne infection has important applications in the study of virulence and immunity.

### TABLE 2

**Specific Infectivity of Different Strains of Tubercle Bacilli**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Animal</th>
<th>Average Number of Tubercles in Lungs</th>
<th>Average Number of Infective Particles Breathed*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ravenel VA</td>
<td>Rabbit</td>
<td>50.0</td>
<td>30</td>
</tr>
<tr>
<td>2</td>
<td>Rabbit</td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>Rabbit</td>
<td>9.0</td>
<td>450</td>
</tr>
<tr>
<td>4</td>
<td>Rabbit</td>
<td>120.0</td>
<td>250</td>
</tr>
<tr>
<td>5</td>
<td>Rabbit</td>
<td>0.5</td>
<td>50</td>
</tr>
<tr>
<td>Human†</td>
<td>Rabbit</td>
<td>1.0</td>
<td>5,000</td>
</tr>
<tr>
<td>Human†</td>
<td>Guinea pig</td>
<td>200.0</td>
<td>5,000</td>
</tr>
</tbody>
</table>

* Estimated by culture from air samples.
† Isoniazid-resistant strain.
From the point of view of the air hygiene study, the important finding was that guinea pigs exposed to drug-resistant organisms developed macroscopic tubercles in their lungs. This suggests that it may be possible to use patients producing drug-resistant organisms as sources of air-borne infection in the next phase of the study.

**Design, Construction, and Operation of a Special Animal Exposure Chamber**

In order to estimate the mean discharge of infective air-borne particles per "open" case of human pulmonary tuberculosis, it is necessary to sample infective droplet nuclei from large quantities of air over a long period of time. For this task the lungs of susceptible animals are far superior to any direct cultural technique. Because of the high dilution of the infective particles in the air and the small respiratory minute volume of a guinea pig, it is expected that it will be necessary to expose 150 to 200 guinea pigs for one year in order to obtain a statistically significant number of infections.

To accomplish this aim, a suitable animal chamber had to be devised (figure 4). A special cage arrangement with a number of unique features was devised by Mr. Wells. Without elaborating on its aerodynamics, its sewage disposal system,
and its continuous water supply, it can be said, on the basis of four months' experience, that animals have remained healthier in the exposure chamber than in ordinary cages. It is assumed that they will not all remain free of tuberculosis when the ward is occupied by infectious patients, for all the special features of the exposure chamber are incidental to the primary purpose, which is to facilitate the uniform exposure of guinea pigs to air from the experimental pilot ward.

**Calibration of the Pilot Ward**

The final step in the demonstration of the suitability of the pilot ward for measurement of infectivity of "open" cases was calibration of the exposure chamber. In this the investigators were helped by Mr. Wells' past experience with a culture having approximately the same specific infectivity as Ravenel VA. (This strain is mentioned as Experiment VII described on page 117 of Mr. Wells' book *Airborne Contagion and Air Hygiene* (1.) A broth culture of Ravenel VA was prepared according to standard technique and undiluted culture filtrate was atomized into the air-conditioning system of the pilot ward. The concentration of organisms in the flask fluid was approximately ten times as high as in the diluted culture filtrate used in Experiment VII cited above. However, the fresh air entering the air-conditioning system was about 100 times greater than that which flowed through the laboratory inhalation chamber used in Experiment VII. The resulting concentration of organisms in the air was thus, by calculation, about one-tenth as great. On the other hand, the rabbits were exposed for thirty minutes in Experiment VII and for two hours in the pilot ward experiment, so that altogether four-tenths as many infective nuclei should have been breathed. In Experiment VII an average of 165 tubercles developed per rabbit, and four-tenths of 165 is 66. Therefore, 50 to 100 tubercles per rabbit were to be expected. As can be seen in table 3 the two rabbits exposed in the pilot ward acquired 61 and 104 tubercles, respectively, and two more animals in the exposure chamber developed 71 and 72 tubercles. These results demon-

<table>
<thead>
<tr>
<th>Ultraviolet Lights in Rooms</th>
<th>Room</th>
<th>Exposure Chamber*</th>
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<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Tubercles per Rabbit</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Off</td>
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<tr>
<td></td>
<td>61</td>
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<td></td>
<td>104</td>
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<td></td>
<td>71, 72</td>
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<tr>
<td>Tubercles per Guinea Pig</td>
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<tr>
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<tr>
<td></td>
<td>28</td>
<td>34, 45</td>
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<td></td>
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<td>26, 22</td>
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<td></td>
<td>7, 29</td>
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</table>

* Exhaust air containing a mixture from all rooms.

? Death before presence or absence of tubercles evident.
strate remarkable control of all of the factors involved in quantitative air-borne infection in the pilot ward.

Another important feature of this experiment was the close approximation between the number of tubercles acquired by animals in the ward and in the exposure chamber. Rabbits in the ward averaged 82 tubercles as opposed to 71 in the exposure chamber, and averages for the guinea pigs were 32 in the ward against 22 in the exposure chamber. This indicated that the air in the exposure chamber was for practical purposes equivalent in infectivity to the air in the ward itself. Furthermore, the rabbits developed on the average about three times as many tubercles as the guinea pigs, as would be expected from the fact that rabbits breathe about three times as much air per minute.

The crucial nature of the information provided by this experiment can be seen from the following calculations. Culture filtrate was atomized at a rate of about three drops per minute, and tubercles were produced in guinea pigs at a rate of approximately 0.2 tubercles per minute. Thus three drops of atomized culture filtrate produced an average of 0.2 tubercles per guinea pig. If 175 guinea pigs had been in the exposure chamber, three drops of atomized culture filtrate would have produced approximately 175 times 0.2 or 35 tubercles. In the next stage of the study when human patients will serve as producers of infective nuclei there will be about 175 guinea pigs in the exposure chamber, and it is hoped to have patients whose sputum contains tubercle bacilli in approximately the same concentration as the culture filtrate which was atomized in this experiment. If the patients' organisms have approximately the same specific infectivity as Ravenel VA, it can be anticipated that something of the order of 35 guinea pigs will be infected by the time the patients have atomized three drops of sputum. The study is planned to run for one year to give ample opportunity for the necessary conditions to be fulfilled. If in one year the patients produce as many infective nuclei as the atomizer produced in one minute, the study will provide a quantitative demonstration of the infectivity of humans with tuberculosis.

In conclusion, attention should be turned from infection to disinfection. From the point of view of air hygiene, the exciting part of the story is the demonstration that experimental animals can be protected against air-borne tuberculosis in a hospital ward. Those of us with a speculative turn of mind wonder whether air hygiene may advance as far in the next half century as water sanitation did in the last half century.

**Summary**

A six-room pilot ward has been adapted to the quantitative study of the infectivity of human patients with “open” pulmonary tuberculosis. The amount of fresh air entering the closed circuit air-conditioning system of the ward was carefully regulated and estimated to show the volume of air into which air-borne particles would be diluted. Each room was equipped with fixtures for irradiating the upper air with ultraviolet light. After artificial contamination of the air with *E. coli*, the effectiveness of the ultraviolet lights in killing the air-borne
organisms was demonstrated. Similar studies were then performed after artificial contamination of the air with bovine tubercle bacilli. A germ-tight ultraviolet envelope surrounded the ward to prevent escape of live organisms into the rest of the hospital. In these experiments animals served as samplers of air-borne infection, the number of tubercles developing in the animals' lungs indicating the approximate number of infective particles inhaled. The effectiveness of ultraviolet lights in killing air-borne tubercle bacilli was demonstrated. A separate experiment showed that a human strain of tubercle bacillus was probably even more infective than the bovine strain when atomized into the air. An exposure chamber to house 180 guinea pigs was then constructed and placed so that exhaust air from the ward passed through it before being vented. The air in this chamber was demonstrated to be nearly equivalent in infectivity to ward air. The pilot ward is now adequately calibrated so that the infectivity of human patients with pulmonary tuberculosis can be estimated from the number of guinea pigs which become infected from breathing air that is exhausted from the ward.

**Resumen**

La Higiene Aérea en la Tuberculosis: Estudios Cuantitativos de la Infectividad y del Dominio en una Sala "Pilota"

Una sala experimental de seis cuartos fue adaptada para el estudio cuantitativo de la infecciosidad de sujetos humanos que padecían de tuberculosis pulmonar "abierta." Graduósé cuidadosamente la cantidad de aire puro que penetraba en el sistema de circuito cerrado de acondicionamiento de la sala, calculándose de modo que mostrara el volumen de aire en que se diluirían las partículas transportadas por el aire. Cada aposento fué provisto de instalaciones destinadas a irradiar la porción superior del aire con luz ultravioleta. Después de la contaminación artificial del aire con E. coli, se comprobó la eficacia de las lámparas de luz ultravioleta para destruir los microbios transmitidos por el aire. Luego se ejecutaron estudios semejantes después de la contaminación artificial del aire con bacilos tuberculosos bovinos. Se circundó la sala con una envoltura ultravioleta a prueba de gérmenes para impedir el escape de microbios vivos al resto del hospital. En estos experimentos, usaronse animales como testigos de la infección transmitida por el aire, indicando el número de tubérculos que se formaban en los pulmones de aquéllos el número aproximado de partículas infecciosas inhaladas. Quedó comprobada la eficacia de las lámparas de luz ultravioleta para destruir los bacilos tuberculosos transmitidos por el aire. Otro experimento demostró que una cepa humana del bacilo tuberculoso era probablemente aun más infecciosa que la bovina al ser pulverizada en el aire. Luego se construyó una cámara de exposición capaz de acoger 180 cobayos, colocándose de manera que la atravesara el aire usado antes de ventilarse. Se demostró que el aire de esta cámara casi equivalía en infectividad al de la sala. La sala experimental está ya calibrada adecuadamente de modo que cabe calcular la infectividad de los sujetos humanos que padecen de tuberculosis pulmonar por el número de cobayos que se infectan al respirar el aire expulsado de la sala.
et déterminée, afin d'évaluer le volume d'air dans lequel les particules transportées par l'air seraient dispersées. Chaque chambre comportait un système d'irradiation de la couche d'air supérieure par l'ultra-violet. Après contamination artificielle de l'air par *E. coli*, l'efficacité germicide des ultra-violets fut démontrée pour les particules dispersées dans l'air. Des études analogues furent alors entreprises après contamination de l'air par des bacilles tuberculeux bovins. Un barrage de rayons ultra-violets, impénétrable pour les germes vivants, prévenait leur passage de la salle expérimentale au reste de l'hôpital. Dans cette expérimentation, des animaux servaient à évaluer la contamination par l'air; le nombre de tubercules se développant dans les poumons de l'animal indiquant approximativement le nombre de particules infectieuses inhalées. L'efficacité germicide sur les bacilles tuberculeux dispersés dans l'air fut démontrée. Une expérimentation distincte a montré qu'une souche de bacille tuberculeux humain nébulisée dans l'air paraissait avoir une contagiosité plus élevée que la souche bovine. Une chambre d'exposition à la contamination pouvant recevoir 180 cobayes fut alors aménagée de façon à permettre à l'air s'échappant de la salle de traverser cette chambre avant ventilation. Il a été démontré que l'air de cette chambre avait un degré de contagiosité sensiblement égal à celui de la salle expérimentale. La salle "pilote" est désormais étalonnée de façon adéquate pour l'évaluation de la contagiosité des malades atteints de tuberculose pulmonaire, selon le nombre de cobayes infectés par inhalation de l'air s'échappant de la salle expérimentale.

**REFERENCE**