Bulletin No. 166 - Sterilization of Brood Combs Infected With American Foulbrood

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The Sterilization of Brood Combs Infected With American Foulbrood

BY C. H. GILBERT

American foulbrood, an infectious disease of the brood of bees, probably known since Aristotle’s time, has for all these years been a menace to beekeeping and is still regarded as the most serious disease with which the beekeeper has to contend. Despite all treatments and methods of control that man has devised to combat this disease, it has gradually spread over Europe and has been introduced into North America, where it has spread to practically all of the beekeeping areas.

The infectious nature of foulbrood diseases has been recognized for many years, but there had been much confusion regarding the causitive organism until 1904, when G. F. White succeeded in isolating and germinating a spore-forming organism which was found to be the cause of American foulbrood. The organism was named Bacillus Larvae, White.

The losses caused by American foulbrood have been exceedingly heavy. During the early history of the disease, its spread was very rapid because beekeepers were not aware of its existence, or did not know methods of diagnosis. As a result, many apiaries were completely wiped out. The beekeepers soon learned that a colony that had died of a brood disease would be robbed out, thus causing the disease to spread. Since no methods of treatment were developed, when diagnosis revealed the presence of American foulbrood, the beekeeper was forced to burn the entire colony. This, of course, caused very heavy losses and many beekeepers began experiments in an attempt to devise methods of combating the disease.

HISTORY

The first relief came when Moses Quinby (11) developed “the shaking plan.” This plan consisted of shaking the adult bees into new hives and the burning or melting of the old combs.
The treatment was described before foundation was known, the bees being given empty wooden frames, or wooden guides. This shaking treatment is used today, as in Quinby’s time, with practically the only improvement being the use of “starters” in the form of full sheets of foundation.

Although the shaking treatment, if properly done, has been a boon to the beekeepers, and they have been able to save hive-bodies, tops and bottoms by scorching them, the loss of combs, which is regarded by many as a serious problem, still confronts them. In an attempt to save the combs, many disinfectants have been tried with varying success. Early in the eighties a phenol cure was tried and recommended (12). However, it was not satisfactory. Water and formalin were used as early as 1890 (5).

Many different solutions were tried, as well as gases, all to no avail, until 1922, when Dr. H. C. Hutzelman (7) developed and placed upon the market a new solution for the disinfection of combs by immersion. Dr. Hutzelman tested the solution in a large way in his own apiary with great success. Samples of treated combs sent to the U. S. Bee Culture Laboratory at Washington, D. C., were pronounced sterile. Concerning this solution, Dr. Hutzelman said: “It will disinfect all sealed brood cells. It will disinfect cells filled with pollen. It will penetrate all propolis on frames. It will completely disinfect all diseased larvae so that these are no longer repulsive to bees. It will disinfect and easily remove scales of dead larvae from cell walls.”

This discovery was enthusiastically received by the beekeepers and many of them prepared to treat combs with the new solution. Barber (4) was the first to try the treatment in a big way. He treated 6,000 combs with striking success. Demuth, after inspecting many of these colonies on treated combs, without finding a cell of foulbrood, wrote his article, “American Foulbrood Ousted” (4). This success stimulated others and many beekeepers began to treat diseased material with the Hutzelman solution.

It was not until September, 1923 (2), that J. L. Byer reported a return of disease upon infected material. Barber (1) criticized Byer’s methods and tried to explain the return of disease
as follows: “1. Failure to dry combs well, thus leaving a cell full of water which would act as a barrier and thus retard sterilization. 2. Use of unguaranteed solution.” Reports of recurrences caused an investigation by the U. S. Bee Culture Laboratory and in March, 1926, a bulletin by Dr. A. P. Sturtevant (15) appeared upon the subject. During the course of this investigation Doctor Sturtevant found that Hutzelman’s solution, along with all other solutions tested, was unsatisfactory, as used, in that it failed to penetrate the cappings of all of the sealed cells. It was, therefore, recommended that all cells be uncapped before treating.

In 1926 E. S. Miller of Indiana (10) said: “Treating with Hutzelman’s solution is, at best, a disagreeable task, and if there are only a few, it doesn’t pay. In a treatment of 5,000 combs recurrences have been few and these we have attributed to overlooked cells of honey.”

In 1926 Byer, a commercial beekeeper of Canada (3), who made thorough field tests of combs treated with Hutzelman’s solution, reported a recurrence of the disease after treatment.

The excessive cost of the alcohol in Hutzelman’s solution made it rather impracticable. Since the alcohol had no disinfecting value, but merely acted as a carrier of the formaldehyde, it seemed desirable to find a cheaper substitute.

The partial success of the Hutzelman solution stimulated interest in disinfection by immersion. In 1923 Prof. H. F. Wilson (21) reported results of a solution known as “Bacilli Kill,” a solution in which sodium hypochlorite was used.

In 1925 R. W. Stratten of Delhi, California (14), reported success with the use of water and formalin. Six hundred treated combs, some of which had been used two seasons, were reported free from disease.

In 1925 G. H. Vansell of California (17) described a new solution of water, formalin and soap and made this statement, “Neither American foulbrood nor the cost of its control need longer handicap the beekeeper and honey producer.”

In 1926 Dr. M. R. Steffen (13) reported partial success with a solution of water, glycerin and formalin.
In 1926 Doctor Sturtevant (15), after testing many disinfecting solutions, made this statement: "A 20 per cent solution of formalin and water was the most satisfactory disinfecting solution for sterilization of infected combs, with regard to both germicidal action and low cost, providing all cells are open and all honey is removed." The results given by Doctor Sturtevant in this report are based upon cultures made in the laboratory and not upon field tests.

In 1928 Jarvis (8), of Ontario, Canada, after conducting experiments with immersion treatment for four years, made the following statements:

1. That alcohol-formalin and formalin water solutions do not always give 100 per cent efficiency where massed spores are concerned.
2. That the treating of brood combs may mean the harboring of disease or even cause it to spread from lack of proper storage facilities."

These conclusions were based upon field tests in which package bees were placed upon treated combs.

There were so many conflicting reports regarding the various treatments of brood combs that the experiments reported herein were conducted in the laboratory and under controlled field conditions in an effort to determine the most effective method of treatment.

**TREATMENTS TESTED**

The following methods of treatment were tested:
2. Water-formalin.
3. Soap bath followed by water-formalin.

*Material used.* All combs used in these experiments were taken from colonies in the last stages of the disease. A large number of cells in each comb contained scales of American foulbrood. In other words, all combs used were "rotten" with American foulbrood. The combs were gone over very carefully to make sure all cells were uncapped, both brood cells and cells containing honey.
The combs were then immersed in a tank of water where they were held until all of the granulated honey was removed from the cells. The length of time required to remove the honey depended upon the amount of granulated honey present. After soaking for a time, each comb was carefully examined under a direct light for sunken cells, malformed cells capped below the surface of other cells, and cells capped with false bottoms. When all cells were uncapped and all of the honey had been removed, the combs were removed from the tank and the water shaken from them. They were then ready for treatment.

Methods of treatment. Combs ready for treatment were placed in a metal rack. This rack was made of angle irons and was, therefore, very rigid. Four chains from each corner of the rack were fastened to a ring in the center, thus permitting the operator to hoist the entire rack out of the solution. The rack was made to hold fifty combs, there being two tiers holding twenty-five combs each. The combs were held in an upright position and metal strips placed along the sides of the tiers prevented the combs from slipping out. This proved to be a convenient way of lifting the combs from the solution. The rack, when submerged, was held in place by weights.

The length of treatment was forty-eight hours in all experiments, except one water-formalin test in which the treatment was continued for seventy-two hours. When the time limit was reached the combs were taken from the disinfecting solution and the solution removed by extraction or shaking. The combs were then rinsed in water under a tap to remove the excess solution, which, if allowed to remain on the combs, causes a disagreeable odor. This is also done to remove the excess formaldehyde so that there would be no formation of paraformaldehyde. The combs were placed in sterile supers which were stacked to permit good ventilation of all combs, thus allowing them to dry rapidly.

Cultures. Yeast extract, egg yolk, agar medium, as described by Sturtevant (16), were used for the cultural tests of the scales taken from the solutions used in treatment. A sterile egg yolk
suspension was prepared, using eggs from hens which had been tested and found free from bacillariarg white diarrhoea. The hens were isolated and eggs taken from the trap nests while warm. The egg was held in a sterile condition, the white drawn off, and the yolk put into suspension without being heated. The yeast extract agar medium was tubed, sterilized, and cooled to 55°C, and the egg yolk suspension was then added to it. The medium was mixed well and slanted and when cool was ready for use. This medium can be held in the refrigerator for months.

The treated combs were aired very thoroughly before cultures were made and all scales were completely dry when placed on the medium. Each scale was removed from the treated comb by means of a sterile needle. The scale was placed in the water of condensation in the tube and allowed to soak until soft. One hour was usually allowed for this. It was then spread over the surface of the medium. Fifteen scales taken at random from each treated comb were cultured. An incubation period of from twenty-four to seventy-two hours was allowed. Two smears were made from each culture. These smears were stained with carbol fuchsin and at least fifteen fields examined under a microscope. Subcultures were made in cases of doubtful germination and, if no growth occurred, a longer period of incubation was allowed. Three untreated scales were cultured as a medium control and two smears made from each culture. Five tubes of medium not inoculated were incubated to check for contamination. Growth on culture medium was regarded as a failure of the solution to sterilize. If no growth occurred on culture medium, the combs were then tested in the field.

Field tests. In the field tests, ten treated combs were used in each hive with package bees placed upon them. These packages were held for several hours in shipping cages before being placed on combs. All colonies were isolated at least ten miles from other bees. Absolute isolation of colonies is possible on the Laramie Plains in the vicinity of the Wyoming Experiment Station, as there are no wild bees in the country and the exact location of every colony of bees in the entire region is known. There is only
one commercial beekeeper in the community and his holdings are not extensive. It is very easy to isolate colonies so that disease conditions can be controlled. The only American foulbrood known to exist in the vicinity is in experimental apiaries under controlled conditions.

The colonies were inspected at twenty-one day intervals during the brood-rearing season. If no infection occurred, the colonies were packed and wintered in the isolated yard and carefully inspected during the second season of brood rearing. If no disease appeared after two seasons, the combs were considered sterilized by treatment. Field diagnosis was checked by microscopical examination of smears prepared from infected material. Foul colonies were removed from the experimental apiaries to a hospital yard, where they were held under observation until treated or destroyed.

**IMMERSION SOLUTIONS USED AND RESULTS OF TREATMENT**

*Hutzelman's Solution.* The solution used was the regular commercial alcohol-formalin solution. Following are the methods and results of each year's experiments in detail:

### 1925

- Number of colonies treated: 4
- Number of combs treated: 40
- Length of treatment: 48 hours
- Bees used: (4 three-pound packages) Italian

**Results**

- Culture medium: No growth.
- Field test: No recurrence of disease.

### 1926

- Number of colonies treated: 4
- Number of combs treated: 40
- Length of treatment: 48 hours
- Bees used: (4 three-pound packages) Italian

**Results**

- Culture medium: No growth.
- Field test: No recurrence of disease.
1927
Number of colonies treated.......................... 4
Number of combs treated..............................40
Length of treatment.................................48 hours
Bees used.............................. (4 three-pound packages) Caucasian

Results
Culture medium: No growth.
Field test: One colony developed American foulbrood.

Soap bath followed by water-formalin. A soap bath was used in this treatment in the proportion of one pound of soap to forty gallons of soft water. The purpose of the soap is to reduce the surface tension of the water and thus get penetration of all cells. Lux flakes, having been used successfully as a reducer of surface tension in previous experiments (6) were also used in the experiment reported here.

When the combs were ready for treatment, they were immersed in the soap bath where they were held for two hours. During this immersion, the soap had very little action upon the combs, except to clean them very well and remove the dirt and debris. The purpose of the soap bath is to get penetration of all cells as quickly as possible. When the soap solution is removed from the combs, a thin film of soap is retained in the cells, thus permitting immediate penetration when the combs are submerged in the disinfectant.

The details of the test of the treatment by means of a soap bath, followed by immersion in water-formalin, follow:

1925
Number of colonies treated.......................... 4
Number of combs treated..............................40
Length of treatment.................................48 hours
Bees used.............................. (4 three-pound packages) Italian

Results
Culture medium: No growth.
Field test: Two colonies developed American foulbrood.
1926
Number of colonies treated: 4
Number of combs treated: 40
Length of treatment: 48 hours
Bees used: (4 three-pound packages) Italian

Results
Culture medium: No growth.
Field test: Two colonies developed American foulbrood.

1927
Number of colonies treated: 4
Number of combs treated: 40
Length of treatment: 48 hours
Bees used: (4 three-pound packages) Caucasian

Results
Culture medium: No growth.
Field test: No recurrence of disease.

*Water-formalin without previous soap treatment.* In this treatment no attention was paid to surface tension. The solution consisted of 80 per cent water (Laramie city water) and 20 per cent formalin. The details follow:

1926
Number of colonies treated: 4
Number of combs treated: 40
Length of treatment: 48 hours
Bees used: (4 three-pound packages) Italian

Results
Culture medium: No growth.
Field test: Two colonies developed American foulbrood.

1927
Number of colonies treated: 4
Number of combs treated: 40
Length of treatment: 48 hours
Bees used: (4 three-pound packages) Caucasian
Results
Culture medium: No growth.
Field test: No recurrence of disease.

1927
Number of colonies treated: 4
Number of combs treated: 40
Length of treatment: 72 hours
Bees used: (4 three-pound packages) Caucasian

Results
Culture medium: No growth.
Field test: One colony developed American foulbrood.

DISCUSSION OF RESULTS
The failure of the disinfecting solutions to sterilize all combs containing scales of American foulbrood is hard to explain. These experiments with brood combs full of scales were very rigid tests of treatment because of the great number of scales present. While all of the cultures made in the laboratory showed no growth of Bacillus larvae, the brood reared in these same combs in the field tests developed the disease in some cases. The small percentage of scales cultured in the laboratory were not sufficient to determine the final results of treatment, because, if given sufficient time, the bees and brood came into contact with one hundred per cent of the scales.

Since every precaution was taken to eliminate any factors, such as the uncapping of all cells, removal of all honey, etc., which would be unfavorable to the complete sterilization of the combs, the failure of the solution to penetrate every cell is left as perhaps the next most important reason the combs were not sterilized.

These factors will always be present in field treatment and, if they cannot be eliminated completely in experimental work under more or less ideal conditions, the question naturally arises, “Will it pay the average beekeeper to take a chance and treat brood combs?” Where there are thousands of scales, each scale containing millions of spores, it may be better to melt the combs, or burn them. This will mean the outright destruction of brood combs, or melting them and rendering the wax.
GAS TREATMENT

The possibility of disinfecting diseased combs by the use of gas was conceived and tried by beekeepers as early as 1900. Since that time many articles relative to the treatment of hives and combs with formaldehyde and other gases, have appeared. Some report satisfactory results, while others report failures. White, in 1903 (18), conducted some preliminary experiments and states in part: "From the experiments made, the conclusion can be drawn that formaldehyde gas is a good disinfectant, but that it penetrates very slowly and that twenty-four hours' application of the gas to the combs, as usually applied, is not sufficient to kill all the spores in decayed larvae."

Again, in 1906, White's (19) work led him to state: "Formaldehyde gas, as ordinarily used in the apiaries, is insufficient to insure complete disinfection."

In 1920 Maassen and Borchert (9) made studies on the efficiency of formaldehyde gas, especially on the spores of American foulbrood. Their conclusion is that the gas does not penetrate well and that it is not sufficient for the disinfection of American foulbrood.

Concerning the Autan process, which is a trade name for a formaldehyde treatment, Maassen and Borchert report the following: "The foulbrood masses were exposed to an extended Autan treatment. They had, despite the extended action of Autan, not lost their ability to transmit the disease. The disease was easily transmitted to healthy colonies by feeding them with honey with which the foulbrood masses had been incorporated. The Autan process, therefore, was again without success. The treatment has failed here completely, as in other cases."

The work of these investigators furnishes evidence that formaldehyde gas cannot successfully be used in the treatment of combs. Treatment with a gas would be so simple and easily carried on, should an effective gas be found, that the writer was prompted to carry on the following experiments with hydrocyanic acid gas.
Methods of treatment with hydrocyanic acid gas. The gas used was generated by the reaction of commercial sodium cyanide, one ounce, commercial sulphuric acid, two ounces, and water, four ounces. The amounts of reagents used in each individual experiment were sufficient for a large volume and, therefore, sufficient for the small disinfectant chamber which was used. The combs were always treated with an excess of the reagent. For the disinfection tests, a heavy aluminum pressure cooker was used. The emergency "pop" valve was replaced by a vacuum gauge, while the pressure gauge was left in place. Vaseline was used on the lid to aid in sealing.

Special attention was paid to all conditions which would favor the penetration of the gas into the combs and scales. The combs used were from hives heavily infected; there were remains of decayed larvae in a large percentage of the cells. All the cells were uncapped. In the various experiments, the combs were placed in horizontal and vertical positions above and below the gas inlet to determine if the position of the comb were a factor in permitting complete penetration of gas. In two tests, in order to insure complete penetration, the combs were held in a partial vacuum for one hour before the gas was admitted. The gas was generated inside the disinfecting chamber in the tests numbered 3, 4 and 5, while in numbers 1 and 2 it was generated outside and piped to the disinfecting chamber.

The combs were cut into three pieces, two pieces being treated; the one not treated was held for a control. One of the treated combs was to be grafted into frames to be used for a field test. The other treated comb was used for tests on culture media. The tests covered from twenty-four to forty-eight hours. The tests were made as follows:

Test Number 1. Combs were placed in the chamber in a vertical position and the lid sealed. A suction pump brought the vacuum indicator to a position which read twenty-three degrees, thus giving a greatly reduced air pressure within the chamber. The valve was closed and the chamber held for one hour. Hydrocyanic acid gas was generated and, as the reaction was very vigorous the
valve was opened and the gas allowed to enter the chamber. When the gauge registered 0.3 lb. pressure within the chamber, the reaction was stopped and the valve closed. The combs were removed forty-eight hours later, wrapped in sterile paper and labeled.

Test Number 2. This test was the same as number one, except that the combs were placed in the treating chamber in a horizontal position and were exposed only twenty-eight hours. The combs were removed, wrapped and labeled as before.

In the above treatments the chamber was taken out in the open air because of the danger from the gas. This change in temperature caused a slight change in the gauge readings, and it was decided to generate the gas inside the chamber and provide a pipe outlet through the window, to act as a safety valve in case of too great pressure and also to allow air to escape when replaced by gas. This was carried out and in the next three tests the chamber was held at a constant temperature.

Test Number 3. In this test the combs were placed in the bottom of the chamber in a vertical position. A beaker was placed above them and the reagents held so that, after sealing the chamber, a slight tilting would cause the sodium cyanide to fall into the solution of sulphuric acid, thus starting the reaction when the chamber was sealed. The outlet was opened and the chamber tilted; the reaction started immediately. The outlet was closed when the gas had replaced the air. The pressure gauge moved up to seven pounds. Twenty-four hours later, when the combs were removed, the pressure gauge registered two pounds. The combs were wrapped and labeled as before.

Test Number 4. This test was made the same as the preceding one, except that the combs were placed in a horizontal position and suspended five inches above the reagents. This was done to insure the complete replacement of air by the gas. The time of exposure was increased to thirty hours. The reaction was carried on as in the preceding experiment. When the outlet was closed the pressure gauge moved up to eleven pounds. Ten hours later
the gauge registered six pounds and when the combs were removed after thirty hours of exposure the gauge registered two pounds. The combs were wrapped and labeled as before.

Test Number 5. The test was the same as the preceding one, except that the combs were suspended in a vertical position above the reagents. The time of exposure was twenty-five hours. After the reaction had started the outlet was closed and the pressure gauge registered eight pounds. Twenty-five hours later, when the combs were removed, the gauge registered two and five-tenths pounds pressure.

Scales treated with hydrocyanic acid gas were cultured as previously described. Cultures were made of scales picked at random over the combs. Three cultures were made from each control comb and fourteen cultures were made from the treated combs.

Results of gas treatment. All cultures of controls gave growth of Bacillus larvae, White. All cultures of treated combs gave growth of Bacillus larvae, White. Growth on every culture proved conclusively that the treatment was not effective and, therefore, no field tests were made. The results of these experiments show that hydrocyanic acid gas is not effective in the sterilization of American foulbrood.

TREATMENT OR DESTRUCTION

The beekeeper, having found American foulbrood in his apiary, is faced with the problem of disposition of infected equipment. If he chooses to destroy the hive and contents by fire, he immediately removes a source of contamination from his apiary and from the community. If he wishes to save the adult bees, he can do so by the shaking treatment, which may be carried on in the apiary or in a hospital yard.

If the colonies are shaken in the yard, there is the danger of spread of disease by drifting of nurse bees and exposures of healthy colonies to infected material. If, on the other hand, the colonies are moved to a hospital yard, there is the danger of the spread of disease incident to moving.
When the adult bees are removed all of the equipment from the original colony must be treated or destroyed. If the beekeeper decides to melt up the combs and render the wax he receives a return for his labor and, if he does it immediately, he has again removed a source of contamination, providing he has carefully destroyed all slumgum and frames and has thoroughly disinfected hive bodies, tops and bottoms.

If, on the other hand, the beekeeper wishes to treat the brood combs, he will then stack the combs to permit the young bees to emerge, thus saving bees and facilitating treatment of the combs.

The practice of stacking infected brood combs and allowing the healthy brood to emerge is of doubtful value. The only value is in ridding the comb of the brood and the saving of the young bees that emerge therefrom. Such combs must be allowed to stand for at least two weeks to permit the young bees to emerge, during which time the combs are poorly protected by bees and there is always a chance for robbing bees to enter. This is especially true when most of the bees are removed from the combs during the shaking treatment. The stacking of many hive bodies together leaves tops, bottoms and supers which must be sterilized or placed in storage immediately. Brood stacked in a yard exposed to livestock may be knocked over and all of the combs robbed by the bees, not only in that yard, but from neighboring yards. Such stacks of brood may also be opened by thieves and left open to marauding bees. When the bees have emerged it is again necessary to shake all of the young bees from the infected combs, thus exposing them to flying bees. The practice of stacking infected brood combs sometimes causes the spread of disease so that the new area of infection is much greater than the original outbreak before treatment.

Brood combs from which the bees have been shaken must be placed in storage until the beekeeper has time to treat them. The winter time is considered most advantageous for this purpose, since most beekeepers do not have time to treat during the honey flow. As a consequence, infected material is held in the honey house during the entire extracting season, thus giving a very good opportunity for the spread of the disease.
When one considers the great chance of spreading the disease by shaking and storage of infected material, the cost of treating solutions and the labor involved, damage to treated combs and, finally, the possibility of a return of the disease on the treated combs, it would be cheaper to melt the combs and render them into wax, or destroy them outright. This depends upon the number of colonies infected with American foulbrood and the number of combs to be treated. If there are hundreds of colonies infected, a small recurrence of disease will not make much difference and the whole treatment can be carried on more economically. If there are only a few colonies involved, it does not pay to take a chance.

Since 1922, when Doctor Hutzelman gave the beekeeping world the alcohol-formalin solution, beekeepers have been treating diseased combs and equipment in a rather large way and with varying success. Many beekeepers felt that it was only a question of time until foulbrood would be a thing of the past. Many articles reporting the success of Hutzelman's solution appeared in the leading bee journals and it seemed that at last brood diseases of bees need not be feared and that there would be no more loss of brood combs. Then came reports of recurrences of disease on treated combs. Many of the most optimistic beekeepers began to lose faith in treatment and the bee journals carried many discussions as to the value of immersion treatments.

After seven years of treatment by immersion, American foulbrood is still a serious problem. It has been reduced a great deal, it is true, but the greatest factor in the control of American foulbrood has been the state inspection work, through educational work and control measures. Many neglected apiaries and hundreds of other sources of infection have been removed. Beekeepers have become better acquainted with bee diseases so that today there are very few apiaries carrying heavy infection. Individual colonies are rarely neglected until they die of the disease, and this was a common occurrence a few years ago.
SUMMARY AND CONCLUSIONS

In the experiments reported herein (a) Hutzelman’s solution failed in one case out of twelve to completely sterilize brood combs infected with American foulbrood after forty-eight hours of treatment; (b) soap bath, followed by water-formalin solution, failed in four cases out of twelve to sterilize brood combs after forty-eight hours of treatment; (c) water-formalin solution failed in two cases out of eight to sterilize brood combs after forty-eight hours of treatment and one case out of four after a seventy-two hour treatment; (d) hydrocyanic acid gas failed to sterilize brood combs when exposed to the gas for twenty-four to forty-eight hours.

From the above results of treatment of brood combs infected with American foulbrood, the following conclusions may be drawn:

1. Unless the technique of treatment of brood combs infected with American foulbrood is improved and made more certain, it is not advisable to treat combs infected with American foulbrood in which scales or decayed larval remains are found.

2. Hydrocyanic acid gas is not effective in the sterilization of combs infected with American foulbrood.

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