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# COMPARISON OF SERUM AND LUNG EXTRACTS FOR SURVEYS OF WILD ANIMALS FOR ANTIBODIES TO *FRANCISELLA TULARENSIS* BIOVAR *PALAEARCTICA*

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ABSTRACT: A comparative study of the antibody titer against *Francisella tularensis* biovar *palaearctica* in serum and lung extract was made using different immunoassays. Samples were taken from experimentally-infected goshawks (*Accipiter gentilis*) and European beavers (*Castor fiber*), and in a field survey of wild beavers. Good accordance between the antibody titer in serum and in lung extract was found in the experimental studies. However, the antibody titer was generally one- to three-fold lower in lung extract than was the titer of serum. Results in the field survey were less reliable. Estimating antibody titer in lung extract is a practical method for surveys for antibody levels, and an alternative to serological surveys, despite lower sensitivity as compared to serum.

*Key words:* Serology, serologic survey, *Francisella tularensis*, tularemia, ELISA, lung extract, antibodies, LEA test, tube-agglutination test, goshawk, beaver, *Accipiter gentilis*, *Castor fiber*.

## INTRODUCTION

Of importance when studying infectious diseases is the prevalence of the disease in animal populations. In domestic animals, this is normally based on serological surveys. Because of the problems associated with blood sampling this is more difficult to achieve in wild animal populations. This problem has been overcome to some extent with the development of new equipment and drugs which facilitate the immobilization of wild animals. Despite this, there are still problems collecting blood from some species owing to anatomical factors (small animals, birds with thin blood vessels, etc.), difficulties with immobilization (access to useful drugs, marine animals, etc.) or other reasons. In these cases, it is necessary to develop new methods for collecting samples for antibody surveys.

Gorin et al. (1979) showed that immunoglobulins can be found in lung lavage fluid. This indicates that the collection of lungs as a source of immunoglobulins is a possible alternative for surveys for antibody levels, which would be especially useful in wild animals, because it is often easier to get samples from dead wild animals than from live ones. A large number of animals are shot every year and lungs could easily be obtained from them. Also, many animals are found dead in the field and sent in for postmortem examination.

Tularemia occurs in a large number of animal species (Hopla, 1974; Jellison, 1974). Many serological surveys for tularemia have been made during recent years (Pearson, 1975; Omland et al., 1977; Long and Clifford, 1978; Binninger et al., 1980; Ferguson and Heidt, 1981; Zarnke and Yuill, 1981; Akerman and Embil, 1982; Mörner and Sandstedt, 1983). In the serological survey for *F. tularensis* in European beaver (*Castor fiber*) (Mörner and Sandstedt, 1983) collection of suitable blood was difficult because of contamination with water. In birds (Mörner and Mattsson, 1987) the main problems were to obtain sufficient amounts of blood and to avoid stress-related mortality during blood sampling.

The purpose of the present study was to investigate whether lung tissue extract from fresh lungs or from lungs stored for several years could be used in surveys for antibody levels for *F. tularensis* in wild animals, using the same method as Waller et al. (1980) have described for encephalitozoonosis. We also compared the titers obtained in serum with those of lung extracts

for sensitivity, reliability and reproducibility.

## MATERIALS AND METHODS

### Hosts

In the experimental studies six goshawks (*Accipiter gentilis*) and four beavers were infected with *Francisella tularensis* biovar *palaearctica* (Mörner and Mattsson, 1987; Mörner and Mattsson, unpubl. data). The animals were infected parenterally (intramuscularly, subcutaneously or intraperitoneally) with 1.0 ml of a suspension of $1 \times 10^7$ bacteria/ml. The animals were killed with an overdose of penthothal sodium, between 14 and 77 days after infection. Lung specimens were taken during postmortem examination, shortly after death.

In the field study 110 shot wild beavers were collected during the years 1974 and 1975. Lungs were removed shortly after death and placed into air tight plastic bags and stored at −20 C until 1982 when the tests were performed.

### Preparation of sera

Blood was collected when experimentally-infected animals were killed. Blood was collected from the heart or chest cavity in shot beavers. Sera were prepared and stored at −20 C. Sera were diluted in phosphate buffered saline solution (PBS), pH 7.2, at 1:20, 1:40, 1:60, 1:80 and then in two-fold dilutions. The sera from wild beavers were diluted at 1:20, 1:50, 1:100, 1:200, 1:500 and 1:1,000.

### Preparation of lung extract

A small piece of lung tissue (approximately 2 $cm^3$) was put in a tube containing 2 ml PBS at pH 7.2, and agitated for 2 min. The lung tissue was removed and the tube was centrifuged at 4,500 rpm for 10 min. The clear supernatant was collected and stored at −20 C. The lung extract was diluted with PBS in two-fold dilutions starting at 1:20.

The preparation of lung extract differed from the method described by Waller et al. (1980) in that sodium azide ($NaN_3$) was omitted since it interfered with the enzyme in the enzyme-linked immunosorbent assay (ELISA).

### Tube-agglutination (TA) test

The tube-agglutination test was performed as previously described by Mörner and Sandstedt (1983) on sera and lung extracts from experimentally-infected goshawks and beavers and on sera from wild beavers.

Heat-treated *F. tularensis* (manufactured by the National Bacteriological Laboratory, S-105 21 Stockholm, Sweden) were used as antigen. The titer was recorded as the reciprocal of the highest dilution that gave visible agglutination.

TABLE 1. Comparison of tube-agglutination test and indirect fluorescent antibody test on serum and lung extract from goshawks (*Accipiter gentilis*) experimentally infected with *Francisella tularensis* biovar *palaearctica*.

| Animal number | Serum | | Lung extract | |
|---|---|---|---|---|
| | TA[a] | IFA[b] | TA | IFA |
| 4100/81 | 0 | 0 | 1:20 | 0[c] |
| 4101/81 | 1:1,280 | 1:1,280 | 1:1,280 | 1:320 |
| 4102/81 | 1:60 | 1:160 | 1:40 | 1:160 |
| 1256/82 | 1:60 | 1:60 | 0 | 1:20 |
| 1891/82 | 1:20 | 1:80 | 0 | 0 |
| 2220/82 | 1:640 | 1:640 | 1:40 | 1:160 |

[a] Tube-agglutination test.
[b] Indirect fluorescent antibody test.
[c] Numbers indicate end-point titer.

### Indirect fluorescent antibody (IFA) test

The IFA test was made on glass slides with formalin-treated whole *F. tularensis* as antigen as described by Mörner and Mattsson (1987). The samples were examined in a Leitz fluorescent microscope with fluorescent attachment incident light and magnitude 400×. The titer was recorded as the reciprocal of the highest dilution that gave a strongly positive FA reaction from the bacteria followed by a weakly positive reaction and secondly by a negative FA reaction.

### Enzyme-linked immunosorbent assay (ELISA)

The ELISA was performed on sera and lung extracts from experimentally-infected beavers and on lung extracts from beavers in the field survey. The ELISA was performed as essentially described by Voller et al. (1978) with modifications as described by Sandström et al. (1986), except that dilute samples of sera and lung suspension were coated on the micro plates. The antigen used was a prepared antigen from a live vaccine strain of *F. tularensis* (Sandström et al., 1984).

## RESULTS

### Experimentally-infected goshawks

Comparison of antibody titers against *F. tularensis* showed good accordance since samples from animals with high serum titers always had extractable antibodies from the lungs (Table 1). The result was similar with both tube-agglutination and the indirect fluorescent antibody test (Table 1).

Antibody titers in lung extract were one-

TABLE 2. Comparison of ELISA, IFA and tube-agglutination test on serum and lung extract from beavers (*Castor fiber*) experimentally infected with *F. tularensis* biovar *palaearctica*.

| Animal identification | Serum | | | Lung extract | | |
|---|---|---|---|---|---|---|
| | ELISA | IFA[a] | TA[b] | ELISA | IFA | TA |
| BARBRO | 1:40,960 | 1:160 | 1:160 | 1:5,120 | 1:40 | 1:20[c] |
| BAMSE | 1:40,960 | 1:1,280 | 1:320 | 1:5,120 | 1:320 | 1:320 |
| BENGT | 1:40,960 | 1:640 | — | 1:160 | 1:40 | — |
| BOSSE (control) | 0 | 0 | 0 | 0 | 0 | 0 |

[a] Indirect fluorescent antibody test.
[b] Tube-agglutination test.
[c] Numbers indicate end-point titer.

to three-fold below those of serum in seven of the 12 tests performed. In one test the titer was four-fold lower in lung extract and in three the result was similar in both tests. In one case (4100/81) an antibody titer of 1:20 was found in lung extract. This titer might be regarded as a non-specific reaction since both the test on serum and the IFA test on lung extract were negative (Table 1).

#### Experimentally-infected beavers

Antibody titers in lung extract and serum of the experimentally-infected beavers also showed good accordance (Table 2). ELISA was the most sensitive method, giving titers up to 1:40,960, while the highest IFA test titer was 1:1,280 and the highest tube-agglutination test titer was 1:320. A generalized infection was present in those beavers with high titers (Mörner and Mattsson, unpubl.).

The difference in titers between ELISA, tube-agglutination test and the IFA test had a range of two- to nine-fold whereas the range in titers between the IFA test and the tube-agglutination test was zero- to two-fold (Table 2). The result of the tube-agglutination test in one beaver (BENGT) was negative both in serum and lung extract. This result was in contrast with the ELISA and IFA test where titers up to 1:40,960 were found (Table 2), and could be explained by obvious bacterial contamination.

TABLE 3. Comparison between serum and lung extract titers in the field survey of beavers (*Castor fiber*) that were positive in any of the tests performed (tube-agglutination, indirect fluorescent antibody, or ELISA) or negative in all three tests; titers < 1:20 are regarded as negative.

| Serum | Lung extract | | |
|---|---|---|---|
| | Positive | Negative | Total |
| Positive | 13 | 45 | 58 |
| Negative | 7 | 45 | 52 |
| Total | 20 | 90 | 110 |

#### Field survey of beavers

The results of the field survey on beavers are listed in Table 3. Of 110 animals tested, both serum and lung extract were positive in 13 (12%) and in 45 animals (41%) both tests were negative (Table 3). Positive serum but negative lung extract were found in 45 animals (41%) and seven animals (6%) had negative serum but positive lung extracts (Table 3).

## DISCUSSION

The purpose of this investigation was to determine whether or not lung extracts contained antibodies in sufficient concentrations to allow surveys for antibodies using this method. We determined also antibody levels in lung extract in comparison with levels in serum. This study shows it is reliable to use lung extracts in surveys for antibody levels for tularemia in wild animals. In the experimental studies, titers generally differed one- to three-fold between serum and lung extract in the tube-agglutination test and the IFA test. This

result is similar to that found by Waller et al. (1980) in their study on rabbit encephalitozoonosis. They also found that the difference in titers between the two methods varied from animal to animal, a finding similar to the results in our study.

ELISA titers were generally several fold higher than titers found with the tube-agglutination test or the IFA test. One explanation for this could be the higher sensitivity of this method owing to the use of a more purified and consequently a more sensitive antigen, as described by Sandström et al. (1984). Moreover, the purified antigen was used for hyperimmunization of rabbits, thereby obtaining specific antibodies for use in the ELISA (Sandström et al., 1986).

The difference in our study between the titers against *F. tularensis* in ELISA and the titers in the tube-agglutination test is similar to results found by Carlsson et al. (1979) in human sera. The median titer in their group of positive sera was 1:4,400 with the ELISA and 1:320 with tube agglutination. This is comparable with our results in the experimental study in beavers, which had a median ELISA titer in serum of 1:40,960 and a median tube-agglutination titer of 1:160 and IFA test titer of 1:640.

The results of the field study in beavers were more varied. Table 3 correlates samples from investigated animals that were positive in any test (TA, IFA or ELISA) and samples that were negative. In 58 animals (53%) the results in the tests were comparable in serum and lung extract (positive/positive or negative/negative). In the remaining 52 animals (47%) the results of the two tests were not comparable. However, there were 45 animals (41%) with positive sera which had negative lung extracts. This is in accordance with the results from the experimental studies, in that animals with low titers in serum gave a non-specific or negative results from lung extract.

The sensitivity of the test of wild beavers was 89% in serum (58 positive serum/65 total positive) and 31% in lung extract (20 positive lung extracts/65 total positive). The lower sensitivity demonstrated in the lung extract antibody (LEA) test could have resulted from several factors. Since concentration of IgG is lower in lung extract than in serum (Gorin et al., 1979) low titers in serum will probably be negative in lung extract. Another explanation for the lower sensitivity in the LEA test could be due to the fact that serum from the wild beavers were tested shortly after the animals were killed, while the lungs were stored deep frozen several years before testing.

Waller et al. (1980) studied extracts of spleen, liver, kidney, and lung. The highest titers were demonstrated in lung extracts. They also demonstrated that the method of detecting IgG in lung extract was reliable even if the carcass had been kept for several days at room temperature before necropsy. They found the highest titers in extracts in six animals dead for 2 days and lowest titers in six animals dead for 7 days.

The specimens used in our field study of beavers had not been dead long before being stored frozen. However, the lungs were stored frozen for several years before testing, which may explain the difference between results obtained in the field survey and in the experimental studies.

The use of the lung extract antibody test has been shown to be a suitable method for studies on tularemia. It could probably be used in other studies on antibody levels in wild animals. The method has obvious advantages in that sample collection is simple and whole animals or collected organs may be stored frozen for a long period before testing. It also allows antibody studies on dead animals submitted for post-mortem examination. It also seems to be a practical method for the wildlife veterinarian who wants to perform surveys on antibody levels of wild animals in species from which it is difficult to obtain blood. Further studies are planned to ascertain whether the described method is reliable for other infectious diseases of wild animals.

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