Leptospiruria in Striped Skunks

EARL E. ROTH, D.V.M., W. V. ADAMS, M.S., G. E. SANFORD, B.A, MARY MOORE, B.S., KAY NEWMAN, B.S., and BETTY GREER, B.S.

THE NATURAL occurrence of leptospirosis in striped skunks, *Mephitis mephitis*, was first reported by McKeever and associates (*1*). They isolated 18 strains from 132 animals collected in Georgia. Ten of these strains were *Leptospira pomona*, five were *L. ballum*, one was not identified, and two were lost. Following this report, additional leptospiral isolates were obtained, some of which were designated as *L. bakeri* by Galton and co-workers (*2*), but were later reclassified as *L. hyos bakeri* by Wolff and Bohlander (*3*). Other strains were found to be a new serotype and were designated *L. atlantae* (*3*). Several strains were found to represent a new subserotype in the hebdomadis serogroup and were designated *L. mini georgia* (*4*).

In preliminary papers, Roth and associates (*5, 6*) reported the occurrence of *L. pomona*, *L. canicola*, *L. ballum*, and members of the icterohaemorrhagiae, hyos, and hebdomadis serogroups among striped skunks in Louisiana. A study in Pennsylvania (*7*) revealed 18 of 54 striped skunks infected with leptospires. Fourteen of the 18 strains isolated in Pennsylvania were *L. pomona*, 3 were *L. ballum*, and 1 was not identified. Additional studies of leptospirosis among skunks in Georgia (*8*) revealed 68 of 430 animals infected. Twelve of the 68 isolates were lost, and the remaining 56 strains were identified as to serogroups as follows: pomona, 31; ballum, 21; hyos, 2; hebdomadis, 1; and grippotyphosa, 1.

Roth and associates (*9*) reported 57.4 percent of 650 striped skunks collected in Louisiana bacteriologically positive for leptospires. *L. pomona* was isolated from 20.9 percent of the animals examined, and *L. hyos hyos* was isolated from 18.9 percent. *L. ballum* was found in 9.7 percent and *L. canicola* was found in 6.2 percent. The serotypes *L. icterohaemorrhagiae* and *L. mini georgia* were found respectively in 2.1 percent and 1.5 percent of the animals. A single strain of *L. grippotyphosa* and a single strain belonging to the australis serogroup were found. Mixed leptospiral infections were detected in 23 animals, the combination of *L. pomona* with *L. hyos hyos* occurring most frequently.

The high incidence of *L. pomona* observed among striped skunks in Louisiana (*9*), Pennsylvania (*7*), and Georgia (*8*) suggests that they may serve as a source of infection to other animals and man. The high bacteriological rate of infection for *L. hyos hyos* found in Louisiana (*9*) suggests that striped skunks may be the primary reservoir of *L. hyos hyos* in Louisiana and possibly in other areas of the United States. The moderate incidence of *L. ballum* and *L. canicola* indicates that the striped skunk may be of secondary epizootiologic importance in regard to these serotypes.

Data on the incidence and types of leptospires are not sufficient to assess the epizootiologic importance of striped skunks in the overall picture of the leptospiroses. In a previous report (*9*) a comparison of the incidence as de-

The authors are with the department of veterinary science, Agricultural Experiment Station, Louisiana State University, Baton Rouge. *Miss Kay Ringer, a laboratory assistant in the department, provided assistance for the studies reported here. The work described in this paper was part of Southern Regional Research Project S–30. The investigation was supported in part by research grant CC 00008–04 from the Communicable Disease Center, Public Health Service.*

termined by bacteriological and serologic methods for each serotype was used to estimate epizootiologic significance. This comparative data revealed that the serologic rate of infection for *L. hyos hyos* among striped skunks in Louisiana was lower than the bacteriological rate. In the case of *L. pomona*, the bacteriological and serologic rates were almost the same. From this data it was concluded that both *L. hyos hyos* and *L. pomona* caused mild infections in striped skunks with little or no mortality, and that infections with these serotypes persisted in the renal tissue for a long period of time.

Studies on the duration of leptospiruria in naturally infected striped skunks were undertaken to obtain additional information on the significance of striped skunks as a potential source of leptospiral infections in man and domestic animals.

Procedures

Collection of animals and basic laboratory procedures have been described previously (*5*, *6*, *9*). Only the procedures pertinent to this report are given in detail here.

Naturally infected striped skunks were employed in this study. Animals whose urine was found positive by darkground microscopy were selected from a population collected at random. They were deodorized surgically while under anesthesia induced by carbon monoxide and ether. No aftertreatment with antibiotics was given. A permanent identification was tattooed on the abdomen of each animal. They were housed individually in specially constructed isolation pens and fed a diet devoid of antibiotics. Each isolation pen was furnished with a nesting box which was also used as a transport cage.

Urine was collected at periodic intervals, essentially as described by Menges and associates (*10*), and cultured for leptospires. The evening before planned collection, the animals were closed in their nest boxes. The skunks were anesthetized with ether, the posterior abdominal area was shaved, and disinfectant (*A*) applied. Urine was aspirated from the bladder with a sterile syringe equipped with a 24-gauge needle. The urine was diluted immediately with nine parts of Stuart's liquid medium and taken to the culture laboratory. Additional dilutions of urine were prepared in Stuart's liquid medium to provide for final dilutions of 1:100, 1:1,000, and 1:10,000. Two tubes of Fletcher's semisolid medium and two tubes of Stuart's semisolid medium were inoculated with 3 to 5 drops from each dilution.

The media were incubated at 30° C. and later examined for leptospires. The first examination was made between 30 and 60 days, and the final examination occurred after at least 75 days. When a specimen was proved positive by darkground microscopy, one tube was retained for identification studies. The remaining tubes were discarded. Negative results were not assessed until all tubes for a specimen proved negative by darkground microscopy.

A leptospiral culture obtained from each positive urine specimen was identified as to serogroup, and representative strains of each sero-

Table 1. Observed duration of leptospiruria in striped skunks from which L. pomona was isolated

Skunk No.	Days cultured after capture and results	Duration in days
317	1(+); 119(+)	119
339	1(+); 80(+)	80
521	1(+); 50(+); 86(+); 120(+); 175(+); 223(−); 273(+); 303(+)	303
583	1(+); 14(+); 48(+); 77(+)	77
631	1(+); 35(+); 61(+); 108(+); 158(+); 189(+); 234(+); 284(+); 321(+)	321
638	1(+); 35(C); 61(C); 109(+); 158(+); 189(+); 234(+); 284(+)	284
639	1(+); 35(+); 61(+); 110(+)	110
645	1(+); 35(+); 61(+); 110(+)	110
767	1(+); 18(+); 67(+); 101(+); 144(+); 195(+); 229(+)	229

NOTE: (+)=positive; (−)=negative; (C)=contaminated.

type were identified by agglutinin-absorption tests. The methods employed have been described previously (*9*).

Results

The results of periodic urine cultures of the striped skunks employed in this study are presented in tables 1 through 5. Table 1 gives the results obtained from animals that yielded only isolations of *L. pomona*, except for three skunks which died after a short period of observation. Seven animals were shown to excrete *L. pomona* in their urine for over 100 days. These results were as follows: skunk 639 shed for 110 days; skunk 645, 110 days; skunk 317, 119 days; skunk 767, 229 days; skunk 638, 284 days; skunk 521, 303 days; and skunk 631, 321 days. None of these animals became negative during the period of observation. Death due to a combination of causes prevented further observations. Consecutive positive cultures were obtained from the urine in all cases except in skunk 521 which gave a negative culture on day 223 but was positive again on subsequent examinations. The second and third cultures on skunk 638 proved contaminated and hence results are not reliable.

Similarly, data on the striped skunks infected with *L. hyos hyos* are given in table 2, except for three animals which were observed positive for 80, 41, and 82 days. The results for the animals tabulated in table 2 show periods of leptospiruria ranging from 123 days to 774 days. Animals 299, 540, and 769 are living while the others have died. Skunk 299 de-

Table 2. Observed duration of leptospiruria in striped skunks from which L. hyos hyos was isolated

Skunk No.	Days cultured after capture and results	Duration in days
299	1(+); 37(−); 147(+); 199(+); 246(+); 311(C); 330(+); 381(C); 417(C); 451(C); 480(C); 506(C); 553(C); 604(C); 621(+);[1] 639(C); 679(C); 729(C); 765(C) 774(+);[1] 844(C); 980(C).	774
540	1(+); 37(+); 73(+); 108(+); 138(+); 163(−); 210(+); 260(+); 291(+); 335(+); 387(−); 422(+); 499(+).	499
557	1(+); 61(+); 92(+); 121(+); 147(+); 196(+); 256(+); 289(+); 331(+); 383(+); 418(+).	418
569	1(+); 22(−); 56(+); 85(+); 111(+); 158(−); 209(+); 214(−); 239(+); 284(−); 334(+); 371(+).	371
572	1(+); 25(−); 57(−); 85(+); 111(−); 159(+); 208(+); 239(−); 284(+); 334(+); 371(+)	371
617	1(+); 21(+); 50(+); 76(+); 123(+)	123
769	1(+); 40(+); 92(+); 126(+); 205(+)	205

[1] Isolation obtained after pretreatment of the animal with sulfathiazole orally.

NOTE: (+)=positive; (−)=negative; (C)=contaminated.

Table 3. Observed duration of leptospiruria in striped skunks from which L. canicola, L. icterohaemorrhagiae, or L. ballum was isolated

Skunk No.	Serotype	Days cultured after capture and results	Duration in days
303	*L. canicola*	1(+); 36(+); 146(+)	146
304	*L. canicola*	1(+); 52(+)	52
562	*L. canicola*	1(+); 16(+); 52(+); 86(+); 115(+); 141(+); 188(+); 239(+); 269(+); 314(+); 364(+); 400(+).	400
578	*L. canicola*	1(+); 20(+)	20
535	*L. icterohaemorrhagiae*	1(+); 42(+); 78(+); 112(+); 142(+); 167(+); 214(−); 264(−); 295(−).	167
680	*L. ballum*	1(+); 47(+); 96(−)	47

NOTE: (+)=positive; (−)=negative.

veloped an infection of the urinary system caused by a gram-negative rod which prevented the isolation of leptospires in pure culture. For 3 days prior to the cultural examinations made on days 621 and 774, this animal was treated orally with sulfathiazole in an effort to reduce the population of the contaminant. A dosage of 1 grain per pound of body weight per day was given in two equal doses. This treatment reduced the population of the contaminant so that leptospires were isolated in pure culture from the higher dilutions of urine but not from the lower dilutions. Consecutive positive results were not always obtained (table 2). Negative results were interpolated between positive results for skunks 540, 569, and 572.

Data obtained on *L. canicola*, *L. icterohaemorrhagiae*, and *L. ballum* are presented in table 3. The longest period of leptospiruria observed for *L. canicola* was 400 days and for *L. icterohaemorrhagiae*, 167 days. Only one skunk infected with *L. ballum* was observed positive for 47 days before death. All these animals died before shedding ceased except skunk 535 which was infected with *L. ictero-*

Table 4. Observed duration of leptospiruria in striped skunks from which L. pomona and L. hyos hyos were isolated

Skunk No.	Days cultured after capture with results and identification of leptospires isolated [1]	Duration in days
315	1(+P); 110(+P); 162(+H); 209(+P); 274(+P); 292(+P)	H=ID P=292
508	1(+P); 53(+P); 89(+P); 123(+H); 153(+H); 178(+H); 225(+H)	H=102 P=89
553	1(+P); 22(+P); 58(+P); 93(+P); 121(+H); 147(+H); 195(+H); 245(+H); 280(+H); 322(+H); 372(+H); 408(+H).	H=287 P=93
576	1(+H); 20(+H); 52(+H); 80(+H); 106(−); 154(+P)	H=80 P=ID
624	1(+P); 21(+P); 50(+H); 76(+P); 124(+P); 179(+H); 204(+P); 250(+H); 299(+H); 335(+H).	H=285 P=204
671	1(+P); 15(−); 63(+H); 112(+H); 144(+H); 189(+H); 241 (+H); 275(+H)	H=212 P=ID
764	1(+H); 16(−); 66(+P); 96(+H); 141(+P); 191(+H); 310(+H)	H=310 P=75

[1] Based on antigenic behavior of the isolate obtained.

NOTE: (+P)=positive for *L. pomona*; (+H)=positive for *L. hyos hyos*; (−)=negative; ID=insufficient data.

Table 5. Observed duration of leptospiruria in striped skunks from which two serotypes were isolated

Skunk No.	Serotypes	Days cultured after capture with results and identification of leptospires isolated	Duration in days
556	*canicola, hyos*	1 (+Can); 23 (+Can); 59 (+Can); 93 (+Can); 125 (+Can); 148 (+H); 196 (−); 252 (+H).	Can=125 H=105
564	*canicola, hyos*	1 (+Can); 31 (+Can); 66 (+Can); 98 (+H); 120 (+H); 168 (+H); 223 (+H); 252 (−); 295 (+H); 353 (+H); 380 (+H); 462 (+NT).	Can=66 H=283
618	*icterohaemorrhagiae, hyos*	1 (+I); 21 (+I); 50 (+H); 76 (+H); 123 (+H)	I=21 H=74
516	*ballum, hyos*	1 (+B); 51 (+H); 87 (+H); 121 (+H); 151 (+H); 176 (+H); 223 (+H); 273 (+H); 304 (+H).	B=ID H=254
585	*pomona, ballum*	1 (+P); 15 (+P); 49 (−); 78 (+P); 104 (+P); 152 (+P); 202 (+P); 232 (+P); 277 (+P); 327 (+B); 363 (+B).	P=277 B=37
588	*pomona, ballum*	1 (−); 15 (+P); 79 (+NT); 104 (+B); 152 (−); 201 (+P)	P=201 B=ID

NOTE: Can=*L. canicola;* H=*L. hyos hyos;* I=*L. icterohaemorrhagiae;* B=*L. ballum;* ID=insufficient data; (NT)=positive, identity not determined, or culture lost; (+)=positive; (−)=negative.

haemorrhagiae. Three consecutive negative cultures were obtained from this skunk before death.

The results on animals from which both *L. pomona* and *L. hyos hyos* were isolated are presented in table 4, except for two skunks that died after short periods of observation. The serotype designation of each isolate was based upon the antigenic behavior of the isolate determined by agglutination tests. Detailed studies of some of these isolates revealed that both serotypes were present even though the strain behaved in agglutination tests as a distinct serotype. For example, strain LSU 3334, isolated from skunk 671 on day 1, behaved originally like *L. pomona*, but underwent a shift in population to *L. hyos hyos* while in liquid medium.

No distinct pattern of shedding is evident in the mixed infections. In skunk 315, *L. pomona* was predominant except in one instance, while in animals 576 and 671, *L. hyos hyos* was predominant except for one isolation. In animals 508 and 553, consecutive isolations of one serotype were obtained, followed by consecutive isolations of the other serotype. However, in skunks 624 and 764, *L.pomona* and *L. hyos hyos* were found alternately.

The results obtained from other combinations of mixed infections are shown in table 5. *L. canicola* and *L. hyos hyos* were observed in skunks 556 and 564, while *L. pomona* and *L. ballum* were observed in animals 585 and 588. Animal 618 yielded isolations of *L. hyos hyos* and *L. icterohaemorrhagiae*, and skunk 516 was positive for *L. ballum* and *L. hyos hyos*.

Discussion

The duration of leptospiruria for certain leptospiral serotypes in striped skunks demonstrated in these studies helps to substantiate the possible epizootiologic significance which was suggested previously (*9*). These observations are particularly meaningful as they were made on animals naturally infected with leptospires. The changing of environment and food posed some problems. Certain animals failed to adjust to captive conditions, while others ate heartily and gained weight.

The period of urinary shedding observed represents the minimum duration. Most of the animals died from various causes before leptospiruria ceased, and they were naturally infected prior to the initiation of these observations. Therefore, the true duration of leptospiruria is longer than the periods reported in this study.

An insufficient number of striped skunks infected with *L. icterohaemorrhagiae* or *L. ballum* were observed in order to evaluate definitely their epizootiologic significance in regard to these serotypes. Six consecutive positive cultures of *L. icterohaemorrhagiae* from skunk 535 revealed an observed duration of leptospiruria for 167 days, but three additional cultural attempts proved negative. It appears safe to conclude that this skunk had ceased shedding *L. icterohaemorrhagiae*. *L. ballum* was observed in one animal which died after 47 days of observation. This single observation of *L. ballum* in one animal does not add any significant information. The single observation of *L. icterohaemorrhagiae* in a striped skunk does, however, suggest a temporary leptospiruria of sufficient duration to serve as a temporary source of infection. The incidence of 2.1 percent for *L. icterohaemorrhagiae* previously reported indicates that the striped skunk is of minor epizootiologic significance for this serotype.

The observation that skunks 303 and 562 shed *L. canicola* in their urine for 146 and 400 days respectively suggests that striped skunks may play an important role in the epizootiology of this serotype. The bacteriological incidence of 6.2 percent previously reported helps to substantiate this hypothesis. Dogs, no doubt, play a major role, but the role of the striped skunk cannot be overlooked in Louisiana. In Israel, van der Hoeden (*11*) considers the jackal as a subsidiary reservoir of *L. canicola*.

Urinary shedding of *L. pomona* was found to be of sufficient duration to further incriminate the striped skunk in the epizootiology of leptospirosis due to this serotype. The high bacteriological rate of infection previously reported for *L. pomona* (*9*) helps to substantiate this conclusion. Four skunks were shown to excrete *L. pomona* in their urine for 229, 284, 303, and 321 days respectively. None of these animals ceased shedding during the period of

observation. The intervention of death due to unrelated causes and the fact that they are infected prior to initiation of these observations necessitates that these periods of leptospiruria be construed as minimum.

The serologic rate of infection for *L. pomona* among striped skunks in Louisiana was reported to be 25.27 percent as compared with a bacteriological rate of 22 percent in the same group of 277 animals (*10*). This criterion indicates that infection of striped skunks with *L. pomona* is a mild nonfatal infection under natural conditions with a high percentage of the animals developing leptospiruria of long duration. The accumulated data on bacteriological evidence, serologic evidence, and duration of leptospiruria preclude ignoring the striped skunk in the epizootiology of *L. pomona* in Louisiana and probably elsewhere. The striped skunk, along with swine and cattle and possibly other animals, supplies the biologic systems necessary for survival of *L. pomona*. Intraspecies and interspecies transmission of *L. pomona* is favored by an abundance of surface water and a continuing susceptible population. Prophylactic measures can be used to reduce the number of susceptible cattle and swine. It is doubtful that *L. pomona* would disappear from the striped skunks in Louisiana even if it were eradicated from swine and cattle.

The observed duration of leptospiruria for *L. hyos hyos* in the striped skunk along with the bacteriological incidence of 19.13 percent and a serologic incidence of 9.39 percent in 277 animals previously reported (*9*) leaves little doubt as to the host-serotype relationship. The higher bacteriological incidence as compared with serologic incidence indicates a minimal immunologic reaction on the part of the host. Furthermore, this criterion suggests that infection of striped skunks with *L. hyos hyos* under natural conditions is a mild nonfatal infection with a long period of leptospiruria following. These studies show a minimum duration of leptospiruria in one animal for 774 days. Four other animals excreted *L. hyos hyos* in their urine for 499, 418, 371, and 371 days. This data on leptospiruria and the data on incidence highly suggest that the striped skunk may be the reservoir of *L. hyos hyos* in Louisiana.

Interestingly, the serotype *L. hyos hyos* has been reported from only one State (Louisiana) in the United States. No clinical signs of disease have been detected in domestic animals due to this serotype and neither have naturally occurring infections in man been reported in the United States. More studies are necessary in regard to *L. hyos hyos* and its occurrence in the United States.

Mixed leptospiral infections were anticipated in striped skunks, but the number observed was somewhat surprising. The combinations of serotypes observed are in accordance with the probability of their independent frequency of occurrence. The combination of *L. pomona* and *L. hyos hyos* was encountered most often, and all other combinations involved either *L. pomona* or *L. hyos hyos* with *L. canicola*, *L. icterohaemorrhagiae*, or *L. ballum*. The seven animals observed infected with both *L. pomona* and *L. hyos hyos* did not exhibit any definite pattern of shedding. The initial isolation of leptospires obtained from skunk 671 behaved in agglutination tests as *L. pomona*. After repeated transfer in Stuart's liquid medium, a population change to *L. hyos hyos* occurred. Antiserum against this mixed culture produced in a rabbit contained antibodies for both *L. pomona* and *L. hyos hyos*.

It is probable that more cultures obtained from the skunks with mixed leptospiral infections contain two serotypes. Some cultures could, however, contain only one serotype, because the dilution of the inoculum (urine) as employed in these studies may result in the separation of serotypes, especially if the population of one serotype greatly exceeded that of the other. More studies, some of which are in progress, are necessary to prove or disprove this hypothesis.

Summary

Duration of leptospiruria in naturally infected striped skunks, *Mephitis mephitis*, was found on the basis of bacteriological evidence to be sufficient to further incriminate striped skunks in the epizootiology of leptospiral infections. *Leptospira hyos hyos* was isolated periodically from the urine of five animals for 774, 499, 418, 371, and 371 days. Similarly,

L. pomona was found in the urine of four animals for 321, 303, 284, and 229 days. Two animals were observed to excrete *L. canicola* in their urine for 146 and 400 days. *L. icterohaemorrhagiae* was isolated from the urine of one skunk over a period of 167 days. The number of days leptospiruria was observed represents minimum periods, because naturally infected animals were studied which died of other causes before leptospiruria ceased.

Some striped skunks were found to be infected with two leptospiral serotypes. The combinations of *L. pomona* with *L. hyos hyos* occurred most frequently. All other mixed infections observed involved either *L. hyos hyos* or *L. pomona* with another serotype.

REFERENCES

(*1*) McKeever, S., et al.: Incidence of leptospirosis in wild animals from southwestern Georgia with a report of new hosts for six serotypes of leptospirae. Amer J Trop Med 7: 646–655 (1958).

(*2*) Galton, M. M., Powers, D. K., McKeever, S., and Gorman, G. W.: The identification of two leptospiral serotypes new to the United States. Public Health Rep 72: 431–435 (1957).

(*3*) Wolff, J. W., and Bohlander, H. J.: Serological classification of five strains of leptospires belonging to the hyos serogroup. Trop Geogr Med 2: 173–179 (1960).

(*4*) Galton, M. M., Gorman, G. W., and Shotts, E. B.: A new leptospiral subserotype in the hebdomadis group. Public Health Rep 75: 917–921 (1960).

(*5*) Roth, E. E., Linder, D., and Adams, W. V.: The use of agar plates as an aid for the isolation of leptospires. Amer J Vet Res 22: 308–312 (1961).

(*6*) Roth, E. E., Adams, W. V., and Linder, D.: Isolation of *Leptospira canicola* from skunks in Louisiana. Public Health Rep 76: 335–340 (1961).

(*7*) Clark, L. G., Kresse, J. I., Marshak, R. R., Hollister, C. J., and Carbrey, E. A.: Leptospirosis in Pennsylvania—a progress report. *In* Proceedings, 65th Annual Meeting of United States Livestock Sanitary Association, pp. 140–146, 1961.

(*8*) Gorman, G. W., McKeever, S., and Grimes, R. D.: Leptospirosis in wild mammals from southwestern Georgia. Amer J Trop Med 11: 518–524 (1962).

(*9*) Roth, E. E., et al.: The bacteriologic and serologic incidence of leptospirosis among striped skunks in Louisiana. Zoonos Res 2: 13–39 (1963).

(*10*) Menges, R. W., Galton, M. M., and Hall, A. D.: Diagnosis of leptospirosis from urine specimens by direct culture following bladder tapping. J Amer Vet Med Assoc 132: 58–60 (1958).

(*11*) van der Hoeden, J.: Epizootiology of leptospirosis. *In* Advances in Veterinary Science, New York, Academic Press, Inc., pp. 277–339, 1958.

SUPPLY REFERENCE

(*A*) Zephiran: Winthrop Laboratories, Inc., New York, N.Y.

Standardized Training for Laboratory Assistants

Laboratory assistants will receive standardized training and national certification under the auspices of the medical profession for the first time. A newly created Board of Certified Laboratory Assistants, sponsored by the American Society of Clinical Pathologists and the American Society of Medical Technologists, is developing a program to provide accredited high school graduates with one year of practical and technical training in routine laboratory work at a level less advanced than that necessary for the professional medical technologist.

After passing an examination given under the direction of the new board, graduates of approved schools for laboratory assistants may place "CLA" after their names. Certification without examination may also be granted at the board's discretion to certificate holders of already established recognized State or regional certifying boards of laboratory assistants. Recognition and approval of this category of laboratory worker by the American Medical Association House of Delegates is expected.

For detailed information and copies of "Essentials" and a "Guide Book" for approved schools of laboratory assistants, write the Secretary of the Board of Certified Laboratory Assistants, Sister M. Rosarii, MT(ASCP), Little Company of Mary Hospital, Evergreen Park, Ill.