FURTHER RESULTS OF SEROLOGICAL EXAMINATION OF DOMESTIC ANIMALS FOR LEPTOSPIROSIS IN AFGHANISTAN

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Abstract. The authors examined serologically 329 specimens of domestic animals from 8 provinces of Afghanistan for the incidence of leptospirosis. They detected in 15.8 % of the animals examined antibodies against Leptospira in titres of 1 : 800 and higher; in 6.4 % with serotypes of the serogroup Hebdomadis, 5.5 % — Tarassovi, 2.7 % — Grippotyphosa, 2.4 % — Pomona, 2.1 % Javanica, 1.6 % — Icterohaemorrhagiae, 0.6 % each — Canicola, Ballum, Bataviae, 0.3 % — Pyrogenes. Positivity was highest in the buffalo — 55.0 %, camel — 10 of the 18 animals examined, and cattle — 25.5 %. It was considerably lower in sheep — 2.3 % and goat — 3.2 %; also positive was one of the 6 zebras examined.

Afghanistan is the least investigated country of central Asia as regards the incidence of leptospirosis. The first reports on the finding of antibodies against leptospira in man and domestic animals have been given by Šebek et al. (1972b). Antibodies against leptospira have not been found in the 929 wild-living animals examined.

In 1974, we had the opportunity to examine serologically for leptospirosis another batch of domestic animals killed at the abattoir of Kabul.

MATERIALS AND METHODS

We examined a total of 329 slaughter animals (for species and number of animals see Table 1) from the provinces Badakhshan, Baglan, Bamyan, Ghazni, Kabul, Kunduz, Nengrahar and Paktija.

Blood from the slaughtered animals was collected on stripes of filter paper and examined in a basic dilution of 1 : 800 with the microagglutination-lysis test (see Šebek et al. 1972a, b) using these serotypes and strains of Leptospira: 1. icterohaemorrhagiae Fryáva, 2. soxil-jalna Sorex Jalna, 3. canicola Canis, 7. 4. arboreae M 7, 5. pyrogenes Salinem, 6. bulgarica Nikolaevо, 7. australis Ballico, 8. jalna Jalna, 9. bratislava Jež Bratislava, 10. pomona Simon, 11. grippotyphosa P 125, 12. hebdomadis Hebdomadis, 13. sejroe M 84, 14. hardjo Hardjoprajitno, 15. wolfi 3605, 16. borincana HS-622, 17. bataviae Van Tienen, 18. tarassovi S 42. For sera giving in a basic dilution a positive reaction with icterohaemorrhagiae, we used parallelly in the examination of titres serotypes copenhageni R. n. 1160, mankanse Mankarse, naam Naam, sarmin Sarmin, birkin Birkin and smithi Smith, for those positive with soxil-jalna serotypes javanica Veldrat Batavia 46, poi Poi, cox Cox and soxia Sofia 874, for those positive with canicola jonasis Jones, sumneri Sumner, bindjoi Bindjoi, schauffneri Vlcrinus 90 C, benjamin Benjamin and malaya H6, for those positive with arboreae ballum Mus 127 and castellonis Castelhon 3, for those positive with pyrogenes zanoni Zanoni, abramis Abraham, biggis Biggs and hamptoni Hampton, for those positive with pomona kennevicki LT 1026, monjakov Monjakov and tropical CZ 299 U, for those positive with grippotyphosa grippotyphosa booth Bernkopf Israel and valbuzzi Valbuzzi, for those positive with hebdomadis or sejroe, hardjo, wolfi and borincana we used in addition to these serotypes worsfoldi Worsfold, medanensis Hord HC, saxkoebing M 24, haemolytica Marsh and ricardi Richardson, for those positive with bataviae paidjan Paidjan, and for those positive with tarassovi we used in addition to this serotype tarassovi (hyos) Mitis Johnson and bakeri LT 79. The final titre was determined for all sera with a positive reaction.
RESULTS

Our results are surveyed in Table 1. Of the serologically positive animals, 1.5% reacted with serotypes of the serogroup *Icterohaemorrhagiae*, 2.1% with those of *Javanica*, 0.6% — *Canicola*, 0.6% — *Ballum*, 0.3% — *Pyrogenes*, 2.4% — *Pomona*, 2.7% — *Grippotyphosa*, 6.4% — *Hebdomadis*, 0.6% — *Bataviae*, 5.5% — *Tarassovi*. Positive reactions have not been obtained with serotypes of the serogroups *Autumnalis* and *Australis*. Sera of 17 animals reacted positively with serotypes of two serogroups, sera of 3 animals with serotypes of even three serogroups. The species with the highest number of positive animals was the buffalo, of which 8 reacted positively with serotypes of two serogroups, two with serotypes of three serogroups. Of the camel, sera of two animals reacted simultaneously with serotypes of two serogroups, one with serotypes of three serogroups. Of the remaining animal species, 5 sera of cattle, one of sheep and one of zebu reacted with serotypes of two serogroups. Since simultaneous positive reactions did not stem from a similarity in the antigenic structure of the pertinent serotypes, it was not coagglutination that was responsible for it, but the immunological response to infection with serotypes of two or three serogroups.

A surprisingly high number of positive cases was obtained for the buffalo (55.0%) and the camel (10 out of the 18 animals examined). It was also high in cattle (25.5%), but low in goat and sheep (3.2% and 2.3% respectively). In the two species with the highest percentage of positivity we detected simultaneous reactions with serotypes of several serogroups: buffalo — 32.5% reacted with serotypes of the serogroup *Hebdomadis*, 22.5% with those of *Tarassovi*, 10.0% — *Pomona*, 7.5% — *Grippotyphosa*, 5.0% each — *Icterohaemorrhagiae* and *Bataviae*, 2.5% — *Canicola*. Of the 18 camels examined, 4 were positive with serotypes of the serogroup *Grippotyphosa*, 3 each — *Icterohaemorrhagiae* and *Pomona*, one each — *Pyrogenes* and *Hebdomadis*. Of cattle, 15.7% were positive with serotypes of the serogroup *Tarassovi*, 13.7% — *Hebdomadis*, 3.9% — *Grippotyphosa*, 2.0% — *Ballum*. For the zebu, a positive reaction was obtained from one of the 6 animals examined; the animal reacted simultaneously with serotypes

| Table 1. Results of serological examination for leptospirosis of 329 domestic animals from the slaughterhouse of Kabul (titre 1 : 800 and higher) |
|-----------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Species        | N   | Posit. | %  | Icte | Jav | Can | Ball | Pyrog | Autumn | Austr | Pom | Grip | Hebd | Bat | Tur |
|----------------|-----|--------|----|------|-----|-----|------|-------|--------|-------|-----|-----|------|-----|-----|-----|
| Sheep          | 88  | 2      | 2.3| 0    | 2   | 0   | 1    | 0     | 0      | 0     | 0   | 0   | 0    | 0   | 0   |
| Goat           | 126 | 4      | 3.2| 0    | 3   | 1   | 0    | 0     | 0      | 0     | 0   | 0   | 0    | 0   | 0   |
| Buffalo        | 40  | 22     | 55.0| 2    | 0   | 1   | 0    | 0     | 0      | 0     | 4   | 3   | 13   | 2   | 9   |
| Cattle         | 51  | 13     | 25.5| 0    | 0   | 0   | 1    | 0     | 0      | 0     | 0   | 2   | 7    | 0   | 8   |
| Zebu           | 6   | 1      | —   | 0    | 0   | 0   | 0    | 0     | 0      | 0     | 1   | 0   | 0    | 0   | 1   |
| Dromedary      | 18  | 10     | —   | 3    | 2   | 0   | 0    | 1     | 0      | 0     | 3   | 4   | 1    | 0   |
| Total          | 329 | 52     | 15.8| 5    | 7   | 2   | 2    | 1     | 0      | 0     | 8   | 9   | 21   | 2   | 18  |

*Icte = Icterohaemorrhagiae, Jav = Javanica, Can = Canicola, Ball = Ballum, Pyrog = Pyrogenes, Autumn = Autumnalis, Austr = Australis, Pom = Pomona, Grip = Grippotyphosa, Hebd = Hebdomadis, Bat = Bataviae, Tur = Tarassovi*
of the serogroups *Pomona* and *Tarassovi*. Of the goat, 2.4% were positive with serotypes of the serogroup *Javanica*, 0.8% with those of the serogroup *Canicola*. Of sheep, 2.3% were positive with serotypes of the serogroup *Javanica*, 1.1% with those of *Ballum*.

As regards the height of the titres, we recorded in the serogroup *Icterohaemorrhagiae* reactions with the serotype *icterohaemorrhagiae* in titres up to 1 : 1,600 in the buffalo and up to 1 : 3,200 in the camel; the serum of one camel reacted with the serotype *naum* in a titre of 1 : 3,200. For *Javanica*, titres in sheep were highest with *sorex-jalna* — up to 1 : 3,200, for goat we obtained titres of the same height with the same serotype, and titres of up to 1 : 6,400 with *poi*; in camel with the serotype *soja* 1 : 3,200. Of the serogroup *Canicola*, the serotype *canicola* in goat attained a titre of 1 : 1,600, 1 : 800 in buffalo, of the *Ballum* serogroup *arborea* in sheep and cattle 1 : 800, of *Pyrogenes pyrogenes* 1 : 800 in the camel, of *Pomona pomona* in titres up to 1 : 3,200 in cattle and zebu, in camel concomitantly *pomona* and *tropica* in a titre of 1 : 1,600, in another animal concomitantly *pomona* and *monjakov* — 1 : 3,200, with *pomona* only 1 : 800. Of the serogroup *Grippotyphosa*, a reaction was obtained with the serotype *grippotyphosa* only: for buffalo in titres up to 1 : 6,400, for cattle 1 : 1,600, for camel up to 1 : 3,200. Of *Hebdomadis*, positive reactions with the serotype *sejroe* in the buffalo attained titres of up to 1 : 6,400, with *saxkoebing* 1 : 6,400, *wolffi* up to 1 : 3,200; in several instances, we found for this serogroup similarly high titres with two, three, and once even four serotypes, up to a height of 1 : 25,000. The highest titre recorded for cattle was given with serotypes *sejroe* and *hardjo* — 1 : 3,200 for both, *borincana* 1 : 800. In the remaining cases, two and once, even three serotypes reacted in titres of the same height. In a camel with serotype *wolffi*, the titre was 1 : 3,200. Of the serogroup *Bataaviae*, the serotype *bataaviae* reacted with a titre of 1 : 1,600 in two bovine animals, of *Tarassovi*, in buffalo and cattle with *tarrassovi* up to a titre of 1 : 6,400, 1 : 800 in zebu with the same serotype.

**DISCUSSION**

While Šebeck et al. (1972b) found serological positivity with two serotypes only — buffalo with the serotype *tarassovi* and a dromedary with the serotype *grippotyphosa* — in an examination of domestic animals from the province Nengrahār, East Afghanistan, our present examination disclosed antibodies against serotypes of the serogroups *Icterohaemorrhagiae*, *Javanica*, *Canicola*, *Ballum*, *Pomona*, *Grippotyphosa*, *Hebdomadis*, *Bataaviae* and *Tarassovi*. A surprising array of serotypes particularly for a country which is mostly arid in character. Earlier results of our leptospirological investigation of wild-living animals from Afghanistan (Šebeck et al. 1972b) suggested that natural foci of leptospirosis dependent on the presence of wild-living animals do apparently not exist in these arid regions and that the only foci existing there are anthropogenic, ie, dependent on domestic animals as their reservoirs. A considerably high percentage of serological positivity for leptospirosis was detected also in domestic animals from arid areas of the Mongolian People's Republic (Šebeck 1974). In the author's opinion, a major role in the maintenance of anthropogenic foci of leptospirosis in arid areas may be played by the watering places of domestic animals. A deficiency of water attracts to these places animals from the surrounding areas to concentrate there, and nearby puddles or even the water source itself may become contaminated with *Leptospira* released with the urine of animals with either acute or chronic infection. In Afghanistan, the serotypes involved in this way in the maintenance of anthropogenic foci of leptospirosis are *pomona*, *tarassovi*, *hardjo* and a type of *grippotyphosa* earlier known under the name *L. bovis*.
The highest percentage of positivity and, simultaneously, reactions with serotypes of the largest number of serogroups have been recorded for the buffalo. The reason may evidently be the close attachment of these animals to water in which they frequently remain for hours. Little information is available in the literature on leptospirosis in the buffalo. A description of its epizootiology has been given for this species from the southern parts of the Soviet Union. Musaev (1959) who examined serologically a herd from these parts found positive reactions with the leptospiral strains Strellok (serotype grippotyphosa) and Giacint (serotype monjakov).

A surprising piece of information was the high percentage of serological positivity, and the demonstration of antibodies against serotypes of six serogroups in the camel. Reports on the detection of leptospiral antibodies in these typical inhabitants of arid areas are scarce in the pertinent literature. Farina and Sobrero (cit. Parnas and Krukowska-Cybulska 1967) found antibodies against L. ballum in a dromedary from Somaliland; Maronpot and Barsoum (1972) found antibodies against Leptospirae (mainly L. grippotyphosa) in a titre of more than 1:128 in 34% of dromedary from Egypt. In Afghanistan, Šebek et al. (1972b) found in this species antibodies against L. grippotyphosa, but in a low titre only (1:800). In 1974, in an examination of 131 two-humped camels from the Mongolian People’s Republic (Šebek 1974), antibodies were detected against serotypes of the serogroup Hebdomadis, with the highest titre of 1:6,400 for the serotype hardjo.

Serological positivity was considerably high in cattle, and positive reactions were given with serotypes of 4 serogroups, but all these had been recorded already for cattle from other countries (Zwierzchowski 1967). In the Mongolian People’s Republic Šebek (1974) found even in arid areas antibodies against Leptospirae in cattle; the average percentage of positivity was 25.0%.

A very low percentage of positivity was found in goat and sheep examined during our present investigation. Positive reactions with serotypes of two serogroups only were obtained for both animal species. Of interest was the detection of antibodies against serotypes of the serogroups Javanica and Canicola. Zwierzchowski (1967) did not report these for sheep and goat; Šebek (1961) found them in goat from Czechoslovakia in titres which were to low to be significant (1:400). In the Mongolian People’s Republic Šebek (1974) demonstrated in 1.56% of sheep leptospiral antibodies in a titre of 1:800; of these in 0.78% against L. sejroe and 0.78% against L. tarassovi; results of his examination of 112 goats were negative.

An understanding of the etiology of the serotype in the individual serogroups may be most important and useful, but no reliable determination could be made on the basis of results obtained from a standard serological examination. Although the highest titre obtained in the individual cases with a certain serotype cannot be regarded as a reliable criterion indicating that infection has been caused by just this serotype, it may be justified to assume, as suggested also by our experience, that this criterion may indicate, in an examination of a larger number of sera, which of the reacting serotypes of a certain serogroups should be the one to be considered. In addition, attention should be given to the geographical distribution of the serotypes which may be involved, the degree of adaptation to a certain host, the incidence of natural reservoirs in the area under consideration, etc. It may be possible to obtain from this aspect an understanding of the etiology of leptospiral serotypes of at least some of the serogroups which have been detected in domestic animals from Afghanistan. In our opinion, the most likely serotype agents of the serogroup Icterohaemorrhagiae is L.icterohaemorrhagiae, although we do not exclude the possibility of existence of L. naum isolated from man in Indonesia (WHO Exp. Group 1967); of Canicola it is L. canicola, of Pomona L. pomona or L. monjakov, of Grippotyphosa L. grippotyphosa, of Bataviae L. bataviae, of Tarassovi L. tarassovi. As
regards the remaining serogroups with positive reactions of their serotypes, it would be illusory to attempt a determination of their serotype etiology.

It is of interest that we did not detect antibodies against serotypes of the serogroups *Autumnalis* and *Australis* in the animals examined in spite of the fact that Šebek et al. (1972b) found antibodies against *L. bratislava* in the sera of persons from the province Nengrahar and from Kabul.

Another important aspect of the epizootology of leptospirosis in domestic animals in Afghanistan which deserves to be mentioned are the characteristics of the natural focality. While the serotypes *pomona*, *tarassovi*, *canicola* and a variant of the serotype *grippotyphosa*, earlier known under the name *L. bovis*, can persist for a prolonged period in populations of domestic animals independent of wild-living animals, the serotypes *icterohaemorrhagiae*, *sorex-jalna*, *poi*, *grippotyphosa* (except for the variant “*bovis*”), *sejroe*, *saxkoebing* and *bataviae* cannot occur and mainly persist without the presence of their natural reservoirs which are wild-living small mammals. The first group forms anthroporourgic foci, the second natural, archaic foci, and this accounts for the completely different character of the epizootology of these two groups of *Leptospira* in domestic animals. This has been pointed out by Šebek (1972) for cattle in Czechoslovakia. Knowledge of the degree of adaptation of several serotypes to domestic animals, and of the time of persistence in these animals without the presence of their natural reservoirs is still incomplete. For example, Roth and Galtón (1960) isolated the serotype *hardjo* from cattle in the U.S.A., Michna et al. (1974) from cattle in Great Britain, but questions of its incidence and importance in natural reservoirs are still unsolved. An interesting piece of information is the fact that *L. hardjo* causes abortion in cattle (Stoenner 1967).

The results of our investigation indicate that foci of the serotypes *icterohaemorrhagiae* and perhaps those of *sejroe* exist apparently in Afghanistan, and that synanthropic rodents, mainly rats of the genus *Rattus* and the house mouse (*Mus musculus*) may act as their possible reservoirs. Šebek et al. (1972 b) obtained negative results from examinations of 124 rats (*121 Rattus rattus, 3 Rattus rattoides*) and from 143 house mice, but these animals had been captured in the province Nengrahar only.

It emerges from the results of our examination of several animal species of Afghanistan that the incidence of leptospirosis may be common in this country and that, evidently, serotypes of several serogroups participate in its etiology. Leptospirosis, being one of the economically important infectious diseases on a world-wide scale (Zwierzchowski 1967), may cause considerable economic losses to Afghanistan. Therefore, increased attention should be given to investigations of this zoonosis in Afghanistan.

ДАЛЬНЕЙШИЕ РЕЗУЛЬТАТЫ СЕРОЛОГИЧЕСКИХ ИССЛЕДОВАНИЙ НА ЛЕПТОСПИРОЗ ДОМАШНИХ ЖИВОТНЫХ АФГАНИСТАНА

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Резюме. Проведено серологическое исследование на лептоспириз 329 домашних животных из 8 провинций Афганистана. Антигена к лептоспирам в титрах 1 : 800 и выше имелось у 15.8 % исследованных животных. Обнаружены серотипы, принадлежащие к следующим серологическим группам: *Heddomadis* — 6,4 %, *Tarassovi* — 5,5 %, *Grippotyphosa* — 2,7 %, *Pomona* — 2,4 %, *Javanica* — 2,1 %, *Icterohaemorrhagiae* — 1,5 %, *Canicola*, *Ballum* и *Bataviae* — 0,6 %, *Pyrogenes* — 0,3 %. Наиболее высокий процент положительной реакции наблюдали у буйвалов (55,0 %), верблюдов (10 из 18 исследованных животных) и скота (25,5 %). Положительная реакция у овец и коз была значительно слабее (2,3 % и 3,2 %); она наблюдалась также у 1 из 6 исследованных зебу.
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