

## The incidence of natural *Clostridium welchii* $\alpha$ -antitoxin in Indian equines: its influence on the results of antigenic stimulus

BY P. N. BASU AND R. N. ROY

*Serum Department, Bengal Immunity Laboratory, Calcutta-36*

(Received 1 January 1963)

The presence of natural circulating antitoxins in horses and its influence on hyperimmunization has been studied by many observers including Bolton (1896), Glenny (1925 *a, b*), Barr & Glenny (1945), Basu & Roy (1946) and Ottenssooser (1946). Bolton (1896), working with 2 horses, failed to observe any correlation between the amount of natural circulating antitoxin and the results of immunization with diphtheria toxin. Other observers mentioned above, working with a much larger number of animals and with different exotoxins, obtained a definite relationship between natural immunity and the immunological performances of the animals on subsequent hyperimmunization, and the significance of the presence of natural antitoxin is now well recognized.

### EXPERIMENTAL

In the present study, natural circulating *Clostridium welchii*  $\alpha$ -antitoxin in 65 young Indian equines—15 horses and 50 mules—was titrated by the lecithovitellin method. Only a single test was done for each animal, shortly after it arrived from north-western parts of India. The mules were from the sub-Himalayan regions and had previously been used as pack and draught animals in hills and towns, while the horses belonged to towns and cities. The end titre in 6 of the mules was not determined but they all contained 1 unit or more per ml. of serum. The titres (units/ml.) observed in the remaining 59 animals are shown in Table 1.

Only 7 of these 59 animals were subsequently immunized for production of *Cl. welchii*  $\alpha$ -antitoxin, and these yielded antitoxin of potency shown in Table 2.

Table 1

	Total number	Number of animals			
		1-2 units	2-3 units	3-4 units	4-5 units
Horses	15	2	6	5	2
Mules	44	2	24	15	3
Total	59	4	30	20	5
		(7%)	(51%)	(34%)	(8%)

Titres determined by lecithovitellin reaction.

Table 2

Serial number of equine	Natural titre (units/ml.)	Titre after immunization (units/ml.)
2026	2	500
2030	4	307
2038	4	307
2039	1	250
2040	1	500
2050	1	166
2062	3	500

Titres determined by lecithovitellin reaction.

## DISCUSSION

Barr & Glenny (1945) working with a larger number of horses observed that natural *Staphylococcus*  $\alpha$ -antitoxin was present in all of them, while only a small proportion of animals had any detectable diphtheria and *Cl. welchii*  $\alpha$ -antitoxins. Ottensosser (1946) observed only 20% of horses in São Paulo had 0.04 unit or more of *Cl. welchii*  $\alpha$ -antitoxin per ml. of serum.

In the present series of 65 equines every animal had 1 unit or more per ml. naturally present *Cl. welchii*  $\alpha$ -antitoxin and about 8% of the animals had 4 units/ml. (Table 1). These figures are very much higher than the figures obtained by previous workers quoted above (Barr & Glenny, 1945; Ottensosser, 1946). Variations in degree and frequency of natural immunity present in animals of different geographical origin have previously been observed (Glenny, 1925*a, b*; Barr & Glenny, 1945; Basu & Roy, 1946). These variations are explained by the difference in environmental circumstances and frequency of natural infection. A cause of such high incidence of natural *Cl. welchii*  $\alpha$ -antitoxin in the present series of Indian animals may also lie in their very special environmental condition, and frequent natural infection.

Antitoxin level in a random sample of natural serum depends on several factors, namely, frequency of and intervals between previous contacts of the animal with the particular antigen, interval between contact and drawing of serum sample for test, and inherent sensitivity of the antibody-producing cells of the particular animal. A single determination of natural immunity cannot reveal fully the immunological possibilities of an animal. Performance test—i.e. response to preliminary antigenic stimulus—is likely to demonstrate potential immunity more correctly. That in the limited number of animals hyperimmunized with *Cl. welchii*  $\alpha$ -toxin the titres obtained were not directly proportional to the natural immunity observed in each of them (Table 2), is, as such, not difficult to explain and is in conformity with previous observations (Barr & Glenny, 1945; Basu & Roy, 1946).

## SUMMARY

Presence of natural *Cl. welchii*  $\alpha$ -antitoxin in 65 Indian equines was observed. All the 65 equines had 1 unit or more of circulating natural antitoxin per ml. of serum. The maximum titre observed was 4 units/ml. in about 8 % of the animals. These figures are very much higher than the figures obtained in other countries. Such a high incidence of natural immunity indicates frequent natural contact with the antigen concerned.

Results of hyperimmunization of 7 equines are recorded and discussed.

Our thanks are due to the authorities of Bengal Immunity Laboratory for permission to publish these observations and to Sri M. Pal for useful technical assistance.

## REFERENCES

- BARR, M. & GLENNY, A. T. (1945). Some practical applications of immunological principles. *J. Hyg., Camb.*, **44**, 135.
- BASU, P. N. & ROY, R. N. (1946). Incidence of natural diphtheria antitoxin in horses: its influence on the results of antigenic stimulus. *J. Hyg., Camb.*, **44**, 348.
- BOLTON, B. M. (1896). Diphtheria antitoxin sometimes found in the blood of horses that have not been injected with toxin. *J. exp. Med.* **1**, 543.
- GLENNY, A. T. (1925*a*). The principles of immunity applied to protective inoculation against diphtheria. *J. Hyg., Camb.*, **24**, 301.
- GLENNY, A. T. (1925*b*). Diphtheria antitoxin in blood of normal horses. *J. Path. Bact.* **28**, 241.
- OTTENSOOSER, F. (1946). Sobre a imunidade natural perfringens em cavalos. Microtitulação dos sôros com a reação de lecithovitulina. *Arch. Biol., S. Paulo*, **30** (275), 111-15. Cited in *Biol. Abstr.* (1947), **21**, 14601.