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The occurrence of salmonellas and lactose-negative Arizonas in reptiles in The Netherlands, and a comparison of three enrichment methods used in their isolation

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SUMMARY

A survey was conducted in 1971 in healthy reptiles supplied to the Central Animal Laboratory of Nijmegen for experimental animal research. In order to determine which salmonella serotypes occur, and whether there are several serotypes per animal, several strains of each positive sample were typed.

It was found that 160 of 169 samples contained salmonellas or lactose-negative Arizonas or both, and 95 different serotypes were isolated.

Of 127 animals examined individually, 67 were carriers of more than one serotype, 42 animals having two types, 21 three types and 4 animals four types.

Three enrichment methods were compared. These were tetrathionate broth incubated at 37° C. (T37) and at 43° C. (T43), and selenite broth incubated at 37° C. (SB). All were incubated for 48 hr. before subculture on brilliant-green agar plates. The enrichment methods T37, T43 and SB produced 99, 125 and 123 positive samples respectively, when taken separately. The combinations of T37 and T43, T37 and SB, and T43 and SB produced 145, 142 and 150 positive samples respectively.

The yield of serotypes in comparable samples showed no difference between the three enrichment methods. With the use of two methods the yield increased by about 38% compared with one method, and the combination of three methods showed an increase in serotype yield of about 64% compared with one method. A distinct preference by serotypes for definite enrichment methods was not proved.

INTRODUCTION

Amongst reptiles, salmonella carriers are found frequently, but the percentage incidences reported in the literature are rather divergent. Hinshaw & McNeil (1947) found salmonellas in 3 of 12 lizards (25%), Lee & Mackerras (1955) in 35 of 52 lizards (67%), Mackey (1955) in 144 of 301 lizards (48%), Bövre & Sandbu (1959) in 27 of 33 tortoises (82%), de Hamel & McInnes (1971) in 28 of 492 lizards (5·7%), whereas Milanov, Chilev, Pashev & Slavkov (1966) found a salmonella carrier rate of 50–100% in tortoises according to their area of origin. In the Netherlands Zwart (1960) and Zwart, Poelma & Strik (1970) found a large number of

types of salmonellas and Arizonas in reptiles which died at zoological gardens. In an earlier survey (Koopman & Janssen, 1972*b*) we found salmonellas in 5 of 10 reptiles.

In order to trace on a larger scale how many healthy reptiles are carriers, and which serotypes they carry, we examined the reptiles supplied to the Central Animal Laboratory of Nijmegen during one year for salmonellas and lactose-negative Arizonas. We also ascertained whether more than one serotype per animal could be demonstrated.

According to Nitzschke (1951), Leistner, Deibel, Johantges & Niven (1962), Mueller (1963) and Luethgen & Lucas (1971) some serotypes sometimes prefer definite enrichment methods. It seemed to us appropriate to check whether such preference could be established for the methods which we used.

MATERIALS AND METHODS

The reptiles examined were supplied to the Laboratory in 1971 on behalf of experimental animal research. One lizard caught in the Netherlands was examined; the rest of the animals were acquired through trade channels and were not of Dutch origin.

A total of 127 animals were examined individually. In addition 42 pooled samples were examined, comprising 12 samples from 2 animals, 3 samples from 3 animals, 14 samples from 4 animals, 12 samples from 5 animals and one sample from 20 animals.

Shortly after arrival of the animals faeces samples were collected. For this, the animals were kept on filter paper. When faeces had been passed the filter paper with faeces was cut out for examination.

The samples were examined for *Salmonella* by the methods described previously (Koopman & Janssen, 1972*a*), but the size of the sample was often much smaller, and varied from 0·1 to 1 g. Each sample was examined by three different enrichment methods; tetrathionate broth at 37° C. (T 37) and at 43° C. (T 43) and selenite broth at 37° C. (SB). After 48 hr. incubation each tube was streaked on two brilliant-green agar plates (BG plates). Suspicious colonies were subcultured to obtain pure cultures (without biochemical examination being done) and sent to the Laboratory for Zoonoses of the National Institute of Public Health, Utrecht, for typing. Usually several strains from BG plates from positive enrichment media were typed.

RESULTS

Of the 169 faecal samples examined, 160 were positive for salmonellas or lactose-negative Arizonas, or both. Of the 127 animals examined individually 119 (93·7 %) were positive. Of the 42 pooled samples, only one sample was negative.

Table 1 shows the results for each species of reptile, and in Table 2 is shown a list of serotypes isolated, together with their animal hosts. Among the 95 serotypes there were 21 which were isolated for the first time in the Netherlands, and one of these types had not been previously isolated (Salm. 30:c, z39 N-group). Table 3 shows the distribution of multiple-serotype isolations among the different samples.

Table 1. *The number of cultures typed and the number of different types of salmonellas and lactose-negative Arizonas per species of reptile*

Species	No. of animals	No. of samples	No. of positive samples	No. of typed cultures	No. of serotypes
Agama stellio	2	2	2	12	2
Basiliscus basiliscus	2	2	2	10	3
Caiman sclerops	30	10	10	36	9
Chamaeleo jacksoni	1	1	1	3	3
Iguana iguana	4	4	4	20	5
Lacerta agilis	1	1	1	3	1
L. galloti	107	34	34	158	29
L. viridis	52	25	23	98	21
Scincus scincus	5	4	4	19	5
Testudo graeca	1	1	1	6	1
T. hermanni	37	37	34	132	31
Tupinambis nigropunctatis	1	1	1	8	2
T. teguixin	53	47	43	190	26
	296	169	160	695	

For a comparison of the three enrichment methods and their possible combinations use was made of the results from 104 comparable positive samples. From a part of these samples two strains per positive enrichment method were typed. Furthermore, these samples include those from which more than two strains were typed, provided that they resulted in the same serotypes. The result of a negative enrichment is denoted by 0; 1 means that the two strains isolated were of the same serotype, and 2 means that they were of different serotypes.

The number of samples from which salmonellas or Arizonas were isolated through one, two or three enrichment methods is shown in column 2 of Table 4. Columns 3 and 4 show the number of strains examined and the number of serotypes found by each method or combination of methods.

Columns 5 and 6 in Table 4 show the number of samples from which two strains per enrichment medium were typed, and the number of different serotypes found in these. Column 7 shows how many different serotypes per method were obtained from 104 comparable positive samples.

Table 5 shows the number of times 0, 1 and 2 serotypes per enrichment method were found from these 104 samples.

Table 6 gives a list of the serotypes isolated, showing the frequency of the number of samples from which these serotypes were obtained through each enrichment method separately.

DISCUSSION

It appears from the results that about 95 % of the reptiles examined individually were established carriers of salmonellas or lactose-negative Arizonas. In the literature such high percentages have been reported for tortoises only. Our high incidence for other species of reptiles is probably partly due to the methods used;

Table 2. *Salmonella types isolated from reptiles in* 1971

S. abony	*Testudo hermanni*
S. abony var. *haifa*	*Testudo hermanni*
S. abaetetuba	*Lacerta viridis*
	Testudo hermanni
	Tupinambis teguixin
S. alachua	*Lacerta galloti*
*S. alger**	*Testudo hermanni*
S. anatum	*Caiman sclerops*
	Tupinambis teguixin
*S. anecho**	*Basiliscus basiliscus*
	Lacerta galloti
	Iguana iguana
*S. argentina**	*Lacerta galloti*
S. baildon	*Agama stellio*
S. ball	*Tupinambis teguixin*
S. bardo	*Lacerta galloti*
	Tupinambis teguixin
	Testudo hermanni
*S. bellville**	*Lacerta viridis*
S. bilthoven	*L. galloti*
	Testudo hermanni
	Tupinambis teguixin
S. bleadon	*Testudo hermanni*
S. canastel	*T. hermanni*
S. carrau	*Lacerta galloti*
S. cerro	*T. hermanni*
S. cubana	*Scincus scincus*
*S. detroit**	*Lacerta viridis*
*S. florida**	*L. galloti*
S. galiema	*Scincus scincus*
S. gaminara	*Lacerta galloti*
	L. viridis
	Tupinambis teguixin
S. give	*T. teguixin*
S. greenside	*L. galloti*
S. halle	*Testudo hermanni*
	Tupinambis teguixin
S. havana	*Chamaeleo jacksoni*
	Lacerta viridis
	Testudo hermanni
	Tupinambis teguixin
S. heidelberg	*Lacerta viridis*
*S. hillbrow**	*L. galloti*
S. houten	*Tupinambis teguixin*
S. hvittingfoss	*Scincus scincus*
	Testudo hermanni
	Tupinambis teguixin
S. infantis	*Caiman sclerops*
S. jodhpur	*Testudo hermanni*
S. johannesburg	*Caiman sclerops*
S. kottbus	*Lacerta galloti*
	L. viridis
	Testudo hermanni
S. langford	*T. hermanni*
S. lindern	*T. hermanni*
*S. luciana**	*Lacerta viridis*
S. marina	*Iguana iguana*
S. midhurst	*Lacerta galloti*
S. muenchen	*Caiman sclerops*
	Lacerta galloti
S. napoli	*L. viridis*
*S. nashua**	*Testudo hermanni*
*S. neasden**	*Lacerta galloti*
S. new-brunswich	*Tupinambis teguixin*
S. newport	*Caiman sclerops*
	Testudo hermanni
	Tupinambis nigropunctatis
	T. teguixin
S. ngozi	*Lacerta galloti*
S. nima	*L. galloti*
	L. viridis
S. oranienburg	*L. galloti*
	Testudo hermanni
	Tupinambis teguixin
S. pomona	*Basiliscus basiliscus*
	Iguana iguana
	Lacerta galloti
	L. viridis
	Testudo hermanni
S. poona	*Tupinambis teguixin*
S. potsdam	*Testudo graeca*
	T. hermanni
*S. pumila**	*Basiliscus basiliscus*
	Iguana iguana
	Lacerta galloti
	L. viridis
	Testudo hermanni
*S. rhône**	*Lacerta galloti*
S. rubislaw	*Testudo hermanni*
S. san diego	*Tupinambis teguixin*
S. saphra	*Tupinambis teguixin*
*S. sendai**	*Testudo hermanni*
S. sheffield	*T. hermanni*
S. siegburg	*Lacerta galloti*
	L. viridis
S. sladun	*Testudo hermanni*
S. sofia	*T. hermanni*
S. sofia var. 27*	*Lacerta agilis*
*S. souza**	*L. viridis*
S. stanleyville	*Scincus scincus*
*S. suelldorf**	*Tupinambis teguixin*
*S. tosamanga**	*Scincus scincus*
*S. tel-hashomer**	*Agama stellio*
S. tennessee	*Testudo hermanni*
S. typhimurium X ORS	*Caiman sclerops*
S. typhimurium I ORS	*C. sclerops*
	Lacerta galloti
S. typhimurium var. Copenhagen XX 652	*L. viridis*
S. typhimurium var. Copenhagen I ORS	*L. galloti*
S. uphill	*L. galloti*
	L. viridis
	Testudo hermanni

Table 2 (*cont.*)

S. vaertan	*Tupinambis teguixin*	*S.* F-group	*Caiman sclerops*
S. vejle	*L. viridis*	*S.* H-group	*Lacerta galloti*
*S. veneziana**	*L. viridis*	*S.* rough	*L. viridis*
*S. virginia**	*L. galloti*		*Testudo hermanni*
S. warragul	*L. galloti*		*Tupinambis teguixin*
S. wassenaar	*Caiman sclerops*		
S. 30:c, z39 (N-group)*	*Lacerta galloti*	*Ar.* 9abc: 24–34	*Lacerta galloti*
		Ar. 5:13–15	*Iguana iguana*
	Tupinambis nigropunctatis	*Ar.* 24:25–26	*Tupinambis teguixin*
	T. teguixin	*Ar.* 1,4:24–38	*Chamaeleo jacksoni*
S. 58a-sub II	*T. teguixin*	*Ar.* 22:1, 2, 5, 6	*C. jacksoni*
S. 44:z4, z23 sub. II	*Lacerta viridis*	*Ar.* 26:32–31	*Tupinambis teguixin*
S. B-group	*Tupinambis teguixin*	*Ar.* 11:16, 17, 18	*Lacerta viridis*
S. C1-group	*Testudo hermanni*	*Ar.* 26:32–21	*Tupinambis teguixin*

* First isolation in The Netherlands.

Table 3. *The division of the positive samples, according to the number of different types of salmonellas and lactose-negative Arizonas per sample**

No. of samples	No. of positive samples	No. of samples with			
		1 type	2 types	3 types	4 types
127 (1)*	119	52	42	21	4
12 (2)	11	4	5	2	0
3 (3)	3	1	2	0	0
14 (4)	14	4	7	3	0
12 (5)	12	3	4	5	0
1 (20)	1	0	1	0	0
169	160	64	61	31	4

* The number of cultures examined per positive sample varies from 1 to 8. The figures in parentheses correspond with the number of animals per sample.

Table 4. *Results of the investigation of* 169 *samples*

Method of enrichment (1)	No. of positive samples (2)	No. of strains investigated (3)	No. of serotypes isolated (4)	Two strains determined		Two or zero strains determined pro enrichment method of 104 positive samples* (no. of serotypes) (7)
				No. of samples (5)	No. of serotypes (6)	
T37	99 (58, 6%)	187	55	69	44	41
T43	125 (74, 0%)	259	61	99	49	44
SB	123 (72, 8%)	269	61	93	51	43
T37+T43	145 (85, 8%)	446	72			57
T37+SB	142 (84, 0%)	456	80			58
T43+SB	150 (88, 8%)	528	85			61
T37+T43+SB	160 (94, 7%)	715	95			70

* A negative enrichment method yielded 'zero' strains.

Table 5. *The number of times that zero, one and two serotypes were found through three enrichment methods in the investigation of zero or two strains of* 104 *comparable positive samples*

No. of serotypes	T 37	T 43	SB
0*	46	22	27
1	46	70	65
2	12	12	12
No. of samples	104	104	104

* Here the enrichment methods were negative, so the yield of serotypes was 'zero'.

none of the investigators mentioned in the introduction used three enrichment methods. There were many different types (95) in our material, and this has been noted by other investigators of reptiles (Boycott, Taylor & Douglas, 1953; Darasse, Le Minor & Lecomte, 1959; Zwart *et al.* 1970). We isolated a number of lactose-negative Arizonas, whereas they are often lactose-positive (Cowan & Steel, 1965). It is therefore possible that Arizonas occur more frequently than appears from our results.

It is remarkable that the animals were often carriers of several serotypes. More than half the animals examined individually harboured more than one serotype. It might be expected that if more cultures per animal were investigated even more serotypes might be found. Previously, the occurrence of several serotypes per animal has only been recorded for tortoises (Boycott *et al.* 1953; Dimow, 1964).

The source from which our animals are bought is the same as for private persons, so that the extent of contamination in reptiles bought as domestic pets will be as great as in our material. In order to trace whether in the Netherlands a relation exists between the serotypes of *Salmonella* found in men and in our reptiles we have compared the 15 serotypes occurring most frequently in the two groups (Table 7). It is seen that only *S. newport* is common to both groups. A number of the serotypes found commonly in reptiles were not isolated from men in 1971, and conversely. In Dutch conditions the risk of human infection from reptiles seems to be less than might have been expected. It is remarkable that an isolation for the first time in the Netherlands, *S. sofia* Var. 27, was from a reptile caught in this country, *Lacerta agilis*.

To minimize the risk of infection for our staff and for the other animals at our institute the reptiles are kept isolated. Up to now this way of housing has been successful.

It appears from Table 4 (column 2) that T43 and SB give much better results than T37. In a previous investigation with dogs and cats it was T37 which proved to be the most satisfactory (Koopman & Jansen, 1972*a*). This shows that it is best to use various enrichment methods in parallel when one desires one standard method only for different animal species. In comparing the three enrichment methods by the yield of the different serotypes (Table 4, columns 5 and 6) there is little difference between the various media. This is striking because considerably

Table 6. *Serotypes isolated and the frequency with which samples from these serotypes were isolated through the various enrichment methods**

Serotypes	T37	T43	SB	Serotypes	T37	T43	SB
S. abony	0	0	1	*S. poona*	0	0	1
S. abony var. *haifa*	0	0	1	*S. potsdam*	3	1	3
S. abaetetuba	4	3	0	*S. pumila*	7	4	8
S. alachua	1	1	0	*S. rhône*	0	0	2
S. alger	0	1	0	*S. rubislaw*	0	0	1
S. anatum	1	6	1	*S. san diego*	1	0	6
S. anecho	3	1	3	*S. saphra*	7	6	8
S. argentina	3	1	6	*S. sendai*	1	1	1
S. baildon	1	1	2	*S. sheffield*	6	5	3
S. ball	0	0	1	*S. siegburg*	2	0	0
S. bardo	3	1	4	*S. sladun*	1	1	1
S. bellville	0	1	0	*S. sofia*	0	1	1
S. bilthoven	2	4	3	*S. sofia* var. 27	1	1	1
S. bleadon	0	1	0	*S. souza*	1	0	0
S. canastel	0	0	1	*S. stanleyville*	0	1	0
S. carrau	0	2	0	*S. suelldorf*	1	1	1
S. cerro	0	0	1	*S. tosamanga*	1	0	0
S. cubana	1	0	1	*S. tel-hashomer*	1	1	0
S. detroit	0	3	4	*S. tennessee*	1	1	1
S. florida	0	1	0	*S. typhimurium* X ORS	0	0	1
S. galiema	1	1	1	*S. typhimurium* I ORS	1	2	0
S. gaminara	3	4	7	*S. typhimurium* var.			
S. give	0	2	1	Copenh. XX 652	0	0	1
S. greenside	0	0	1	*S. typhimurium* var.			
S. halle	1	4	5	Copenh. I ORS	0	1	0
S. havana	6	6	1	*S. uphill*	0	0	2
S. heidelberg	0	1	0	*S. vaertan*	1	0	0
S. hillbrow	1	0	1	*S. vejle*	0	1	0
S. houten	0	0	2	*S. veneziana*	0	0	1
S. hvittingfoss	2	2	2	*S. virginia*	0	0	1
S. infantis	0	1	0	*S. warragul*	1	0	0
S. jodhpur	0	3	0	*S. wassenaar*	1	1	0
S. johannesburg	0	1	0	*S.* 30:c, z39 (N-group)	2	3	3
S. kottbus	1	6	1	*S.* 58a-sub. II	0	1	0
S. langford	1	0	0	*S.* 44:z4, z23 sub. II	0	0	2
S. lindern	1	0	1	*S.* B-group	0	0	1
S. luciana	1	0	1	*S.* C1-group	1	1	0
S. marina	1	0	1	*S.* F-group	0	1	0
S. midhurst	1	1	1	*S.* H-group	0	1	0
S. muenchen	1	3	1	*S.* rough	3	0	0
S. napoli	1	0	2	*Ar.* 9abc:24–34	0	0	1
S. nashua	1	0	0	*Ar.* 5:13–15	0	1	1
S. neasden	1	2	1	*Ar.* 24:25–26	0	1	4
S. new-brunswich	2	2	1	*Ar.* 1, 4:24–38	0	0	1
S. newport	5	10	3	*Ar.* 22:1, 2, 5, 6	0	0	1
S. ngozi	4	3	1	*Ar.* 26:32–31	1	1	0
S. nima	3	4	0	*Ar.* 11:16, 17, 18	2	2	5
S. oranienbrug	7	10	6	*Ar.* 26:32–21	0	0	2
S. pomona	7	16	11				

* A negative enrichment method yielded 'zero' strains.

Table 7. *Comparison of the* 15 *Salmonella serotypes occurring most frequently in men in The Netherlands and the* 15 *serotypes occurring most frequently in reptiles at the Central Animal Laboratory, Nijmegen, in* 1971

	Salmonella serotypes isolated from men		Salmonella serotypes isolated from reptiles	
1	*typhimurium*	(+)*	*pomona*	(+)†
2	*panama*	(−)	*saphra*	(−)
3	*infantis*	(+)	*oranienburg*	(+)
4	*enteritidis*	(−)	*newport*	(+)
5	*brandenburg*	(−)	*pumila*	(−)
6	*heidelberg*	(+)	*gaminara*	(+)
7	*stanley*	(−)	*havana*	(+)
8	*thompson*	(−)	*halle*	(−)
9	*derby*	(−)	*sheffield*	(−)
10	*agona*	(−)	30:c, z39	(−)
11	*newport*	(+)	*argentina*	(−)
12	*eimsbuettel*	(−)	*bilthoven*	(−)
13	*montevideo*	(−)	*sandiego*	(+)
14	*bredeny*	(−)	*detroit*	(−)
15	*muenchen*	(+)	*bardo*	(−)

* +, Isolated from reptiles; − not isolated from reptiles (1971).
† +, Isolated from men; −, not isolated from men (1971).

fewer T37 samples were concerned in the comparison than samples of the other enrichment media.

A total of 104 samples could be used for a direct comparison of the number of serotypes found with the three enrichment methods and their combinations. It appears from this that there is little difference between the three methods. The yield increases as more enrichment methods are used in parallel. It is to be expected that the use of more than three enrichment methods would give a further increase in yield, but this increase would probably not counterbalance the additional work and materials.

When, in the case of the 104 samples, we consider how many times an enrichment method is negative, and how many times one or two serotypes were found from the two isolated strains, it appears that T37 gives the poorest yield, in the sense that this method was the most negative. This was to be expected because this method, in the total results, also showed the lowest yield of positive results.

Although it appears that the affinity for a single type to the three enrichment methods was not equal every time, it was not possible to find a preference of certain serotypes for one of the three enrichment methods.

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