Minimum Inhibitory Concentrations of Different Antibiotics for Pasteurella Multocida, Pasteurella Haemolytica and Corynebacterium Pyogenes of Bovine Origin, and Therapeutic Considerations

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A wide range of microorganisms with varying pathogenicity has been isolated from the lungs of cattle affected by enzootic bronchopneumonia (transport pneumonia, shipping fever); nevertheless there is no doubt that *P. haemolytica* and *P. multocida* are the main causes of this disease. In the advanced stage of the condition *C. pyogenes* is frequently also present as a secodary invader. It is therefore of practical and therapeutic significance to regularly review the sensitivity of these bacteria to the antibiotics available for the treatment of pneumonia.

Materials and Methods

Nasal swabs (n = 392) were taken from the airways of calves which were afflicted by, or had died of enzootic bronchopneumonia (EBP), as well as from calves which were initially healthy but later became affected by EBP. The samples were examined for the above-mentioned organisms by the procedure described previously[8] and the minimal inhibitory concentration (MIC) of the following antibiotics for 22 strains of *P. haemolytica* (P.h.), 50 strains of *P. multocida* (P.m) as well as for 15 *C. pyogenes* (C.p.) strains were determined: amoxycillin trihydrate, oxacillin sodium, neomycin sulphate, kanamycin sulphate, spectinomycin sulphate, tylosin tartrate, spiramycin, lincomycin hydrochloride, oxytetracycline hydrochloride and also a chinoloncarbonic acid ester (Baytril-Bayer) (only for C.p.).

Results

Total number of isolated bacterial types

From the 392 swab samples the named organisms were isolated in the following distribution:

P. multocida — 121 strains
P. haemolytica — 74 strains
C. pyogenes — 15 strains

In 38 swab samples *P. multocida* as well as *P. haemolytica* were detected.

The *minimum inhibitory concentrations* of the tested antibiotics for the isolated germs are listed in the following two tables.

Table 1: Minimum inhibitory concentrations of the tested antibiotics for 50 strains of P.multocida and 22 strains of P.Haemolytica

mg/l	0.125	0.25	0.5	1	2	4	8	16	32	64	128	>128
Amoxicillin												
P.h.	4	10	2							1	2	3
P.m.	45	5										
Oxacillin												
P.h.					1				14			7
P.m.	7	6	22	8	2	2		1	2			
Neomycin												
P.h.						1	7	14				
P.m.				5	14	16	14	1				
Kanamycin												
P.h.						7	15					
P.m.			5	9	13	21	2					
Spectinomycin												
P.h.								20	1			1
P.m.							6	44				
Tylosin												
P.h.									13	9		
P.m.				3	5	7	12	8	14	1		
Spiramycin												
P.h.										3	16	3
P.m.						4	7	5	11	19	3	1
Lincomycin												
P.h.								2	9	11		
P.m.							1	9	36	4		
Oxytetracyclin												
P.h.					12	2				5	3	
P.m.			1	2	12	11	13			6	5	

Table 2: Minimum inhibitory concentrations of the tested antibiotics for 15 strains of Corynebacterium pyogenes.

mg/l	0.125	0.25	0.5	1	2	4	8	16	32	64	128	>128
Amoxicillin C.p.	15											
Oxacillin C.p.		2	7	6								
Neomycin C.p.							10	5				
Kanamycin C.P.					8	7						
Spectinomycin C.p.			3	11			1					
Tylosin C.p.	5	5	1	1						1	1	1
Spiramycin C.p.		1	9	1					1			3
Lincomycin C.p.		4	6	1							1	3
Oxytetracyclin C.p.					5		2	3	5			
Chinoloncarbon-säure-Ester C.p.				9	5	1						

Discussion

Of initial interest is the high proportion of *P. haemolytica* in the Pasteurella isolates, that is, 38%. A higher pathogenicity is ascribed to P. haemolytica than to P. multocida; in addition the organism is, as these results also show, less sensitive to most antibiotics and chemotherapeutic agents.

In evaluating the therapeutic usefulness of a drug from the MIC values must not only the size and duration of the active agent concentration in a feasible dosage be considered, but also a number of other influencing factors such as degree of binding to protein, tissue concentration in the lungs, pH dependency, bacteriostatic or bacteriocidal method of action, post-antibiotic effect, inhibiting fibrin barriers etc. The establishing of criteria for evaluating a compound is therefore restricted by uncertainties. Nevertheless the gradings based on the MIC values have proved to be valuable guidelines for practitioners.

Bearing in mind the provisos listed above, the antibiotics which were tested can be assessed as follows (in line with the recommendations of the Medical Standards Committee (1981) the effective agent concentration is taken as that reached in serum half-way through an application period at the recommended dosage):

Amoxycillin: at a dosage of the trihydrate of 7 mg/kg/die i.m. a limit value of 1 mg/l is obtained, at which 92% of the Pasteurellae and all C.p. strains should be inhibited. *(Fig. 1)*

Kanamycin: dosage 10 mg/kg i.m. at 8-hours intervals, giving limit value of 4 mg/l → should inhibit 75% of the Pasteurellae and 100% of the C.p. *(Fig. 2)*

Spectinommycin: 20 mg/kg i.m. at 8-hours intervals → limit value of 8 mg/l → should inhibit all C.p. strains but merely 8% Pasteurellae.
Dosage 30-40 mg/l → limit value of 16 mg/l → would inhibit 97% of the Pasteurellae.

Oxacillin: dosage 25 mg/kg i.m. at 12-hours intervals → Limit value 0.5 mg/l → 50% of Pasteurellae (only P.m.) inhibited and all C.p. strains. *(Fig. 3)*

Neomycin: doses of 12 mg/kg or 22 mg/kg i.m./s.c. at 12-hours intervals → limit value 4 mg/l or 8 mg/l → 50%/80% Pasteurellae inhibited; only 8 mg/l inhibit 66% of C.p. At 22 mg/kg already risk of toxicity. Better effect on intratracheal application? *(Fig. 4)*

Oxytetracycline-HCl: dosage 11 mg/kg/die i.v./i.m./s.c. → limit value 2 mg/l → 38% of Pasteurellae inhibited, 33% C.pyogenes.

Provided that the limit value in the lungs is 4 mg/l → 55% Pasteurellae inhibited. Significantly higher concentration on intratracheal administration? *(Fig. 5)*

Tylosin: dosage 10 mg/kg/die i.m. should inhibit 2/3 of the C.p. strains for sevral hours, not however the Pasteurellae. Increasing the dosage to 20 mg/44 mg/kg or intratracheal administration (50 mg Tylosin-base/kg) do not result in a significant increase in effectiveness. *(Fig. 6)*

Spiramycin: dosage 50 mg/kg/die → limit value 2 mg/l → inhibits approx. 11/15 of the C.p. strains, but not the Pasteurellae. *(Fig. 7)*

Lincomycin: dosage 10 mg/kg i.m. in 12-hours intervals → limit value 2 mg/l → should inhibit 11/15 C.p. strains, but not the Pasteurellae. *(Fig. 8)*

Conclusions

The results show that *P. haemolytica* and *P. multocida* proved to be very often sensitive to amoxycillin, often sensitive to kanamycin and only relatively high concentrations sensitive to neomycin and spectinomycin; both bacteria were frequently resistant to oxacillin and oxytetracycline and always resistant to tylosin, spiramycin and lincomycin. *C. pyogenes* proved to be always sensitive to amoxycillin, oxacillin, kanamycin and spectinomycin, frequently sensitive to tylosin, spiramycin and lincomycin, frequently resistant to oxytetracycline and neomycin. Considering the results of the in vitro testing as well as the achievable drug concentrations in vivo amoxycillin and kanamycin seem to be good candidates for the treatment of EBP. With the other drugs tested different restrictions have to be considered.

Summary

For 50 strains of *Pasteurella multocida* (P.m.), 22 strains of *Pasteurella haemolytica* (P.h.) and 15 strains of *Corynebacterium pyogenes* (C.p.), isolated from the

Figure 1: Serum concentration of Amoxycillin in healthy calves after i.m. application of different doses (YEOMAN, 1977) in relation to the minimum inhibitory concentrations for Pasteurella multocida (50 strains), Pasteurella haemolytica (22 strains) and Corynebacterium pyogenes (15 strains).

× = one strain of Pasteurella haemolytica
\+ = one strain of Pasteurella multocida
o = one strain of Corynebacterium

A: Amoxycillin-Na 7mg/kg
B: Amoxycillin trihydrate 7 mg/kg
C: Amoxycillin trihydrate 14 mg/kg

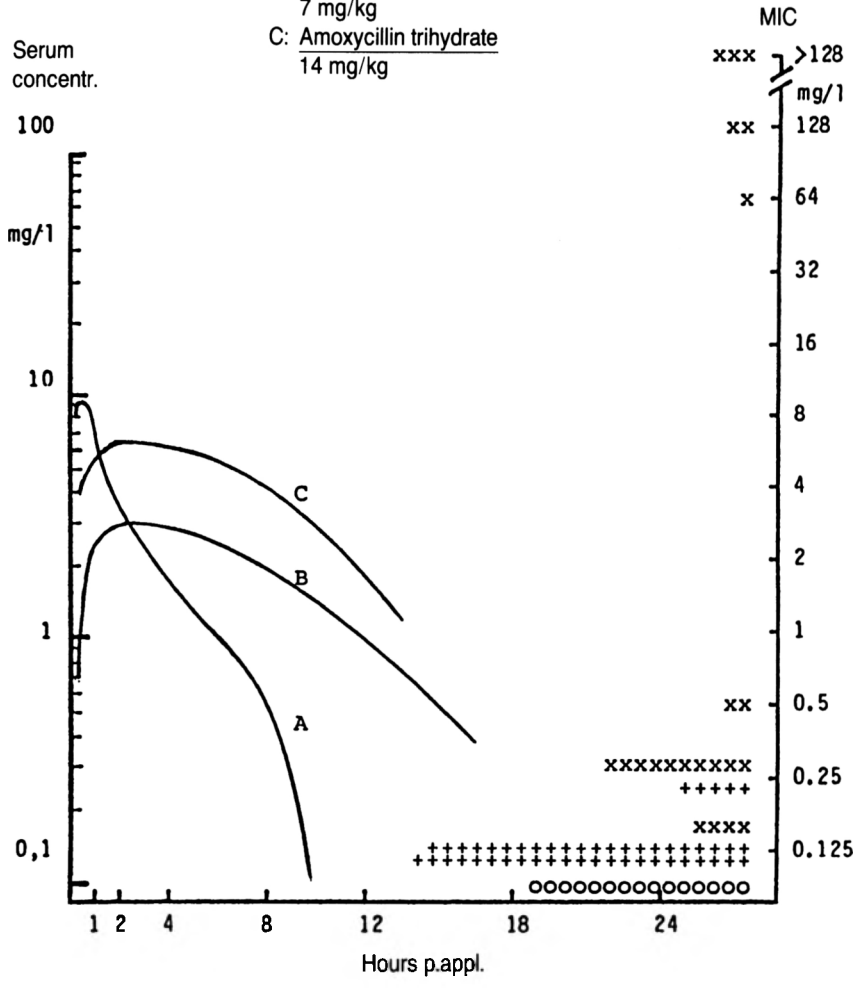


Figure 2: Serum concentration of Kanamycin sulphate in healthy calves after s.c. application of different doses (HJERPE and ROUTEN, 1976) in relation to the minimum inhibitory concentrations for Pasteurella multocida (50 strains), Pasteurella haemolytica (22 strains) and Corynebacterium pyogenes (15 strains).

× = one strain of Pasteurella haemolytica
\+ = one strain of Pasteurella multocida
o = one strain of Corynebacterium

Kanamycin sulphate

A: 11 mg/kg
B: 55 mg/kg

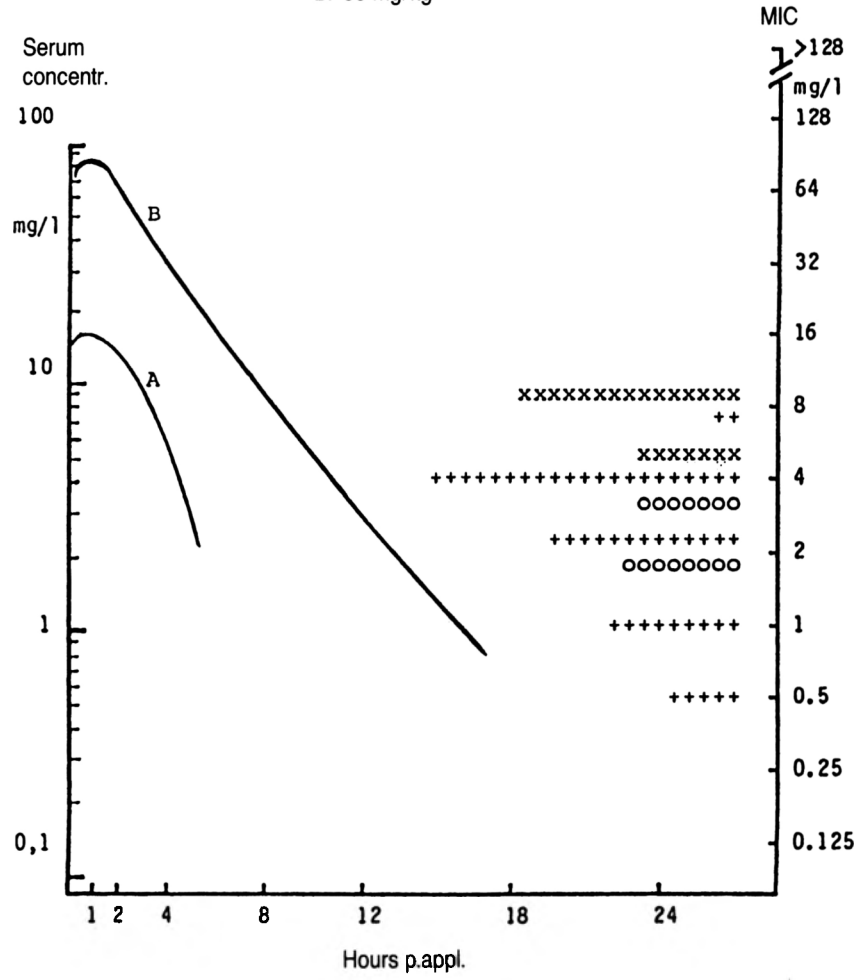


Figure 3: Serum concentration of Spectinomycin in healthy calves after i.m. or i.v. application of a dosage of 20 mg/kg (ZIV and SULMAN, 1973) in relation to the minimum inhibitory concentrations for Pasteurella multocida (50 strains), Pasteurella haemolytica (22 strains) and Corynebacterium pyogenes (15 strains).

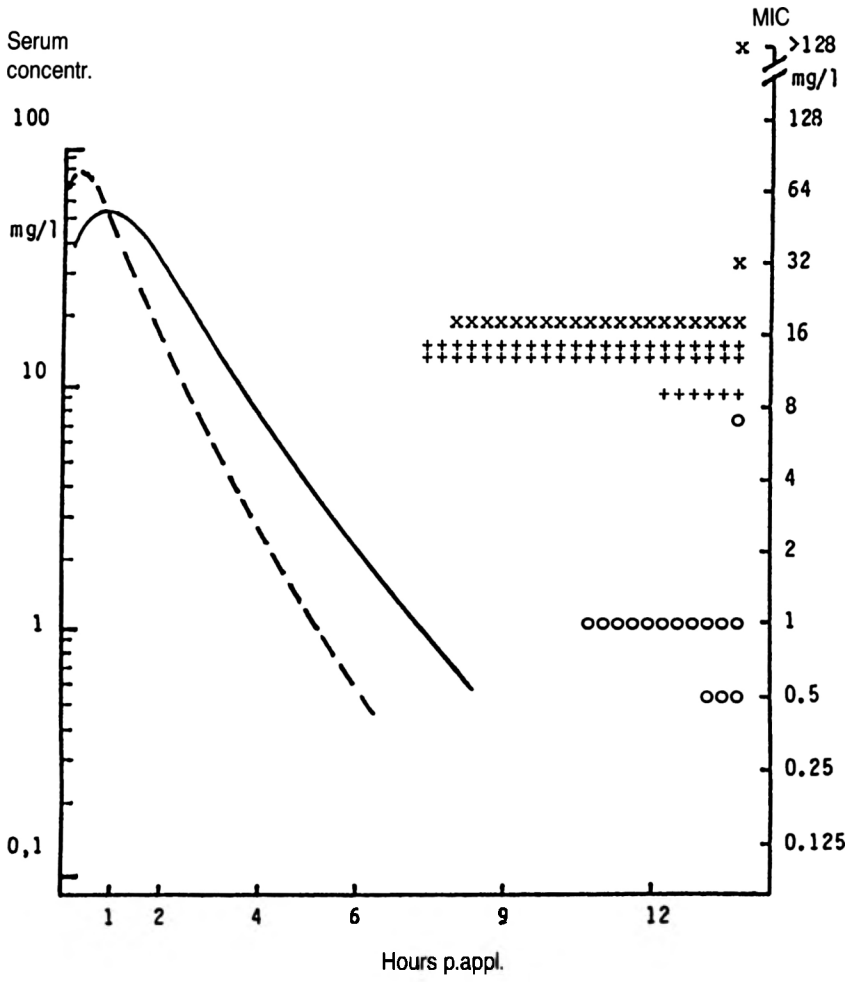


Figure 4: Serum concentration of Neomycin sulphate in healthy calves after i.m. and s.c. application of different doses (HJERPE and ROUTEN, 1976; BLACK et al., 1983) in relation to the minimum inhibitory concentrations for Pasteurella multocida (50 strains), Pasteurella haemolytica (22 strains) and Corynebacterium pyogenes (15 strains).

× = one strain of Pasteurella haemolytica
\+ = one strain of Pasteurella multocida
o = one strain of Corynebacterium pyogenes

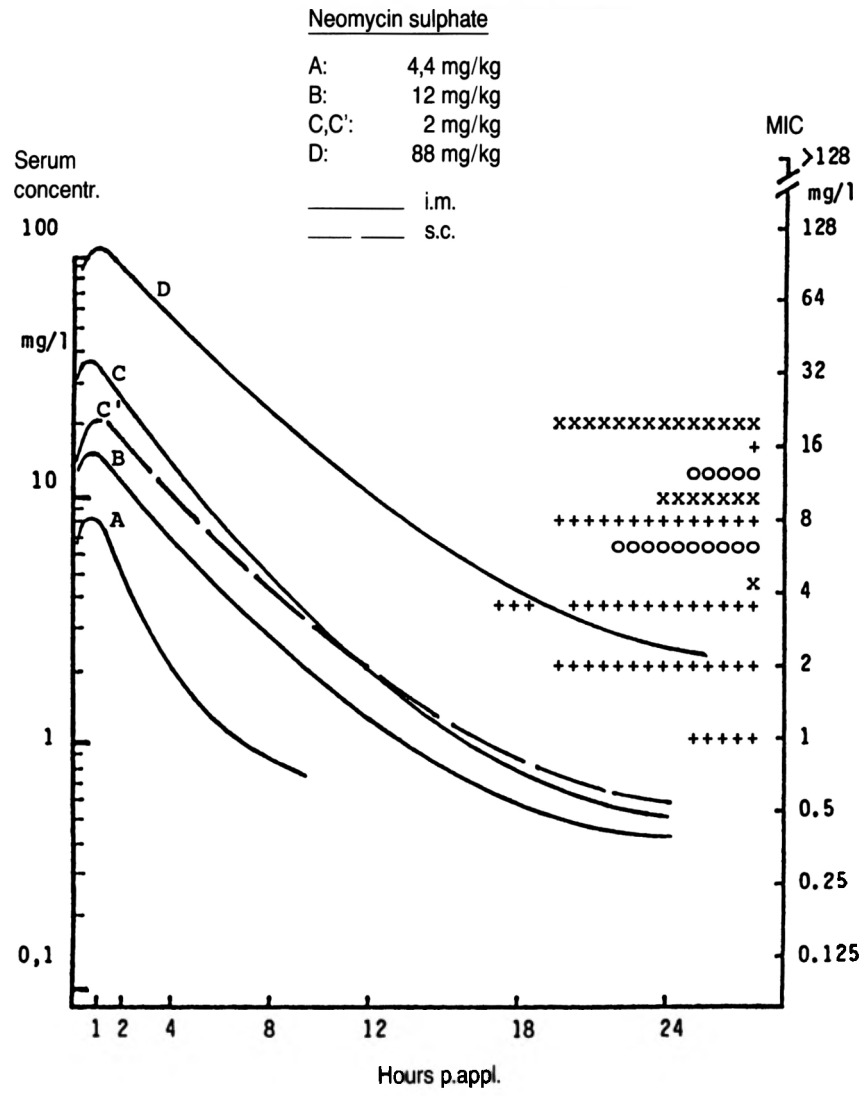


Figure 5: Serum concentration of Oxytetracycline hydrochloride in healthy calves after i.m. application of different doses and preparations (TOUTAIN and RAYNAUD, 1983) in relation to the minimum inhibitory concentrations for Pasteurella multocida (50 strains), Pasteurella multocida (22 strains) and Corynebacterium pyogenes (15 strains).

× = one strain of Pasteurella haemolytica
\+ = one strain of Pasteurella multocida
o = one strain of Corynebacterium pyogenes

A: OTC-dihydrate (long acting) 20 mg/kg
B: OTC-HC1 10/5/5 mg/kg

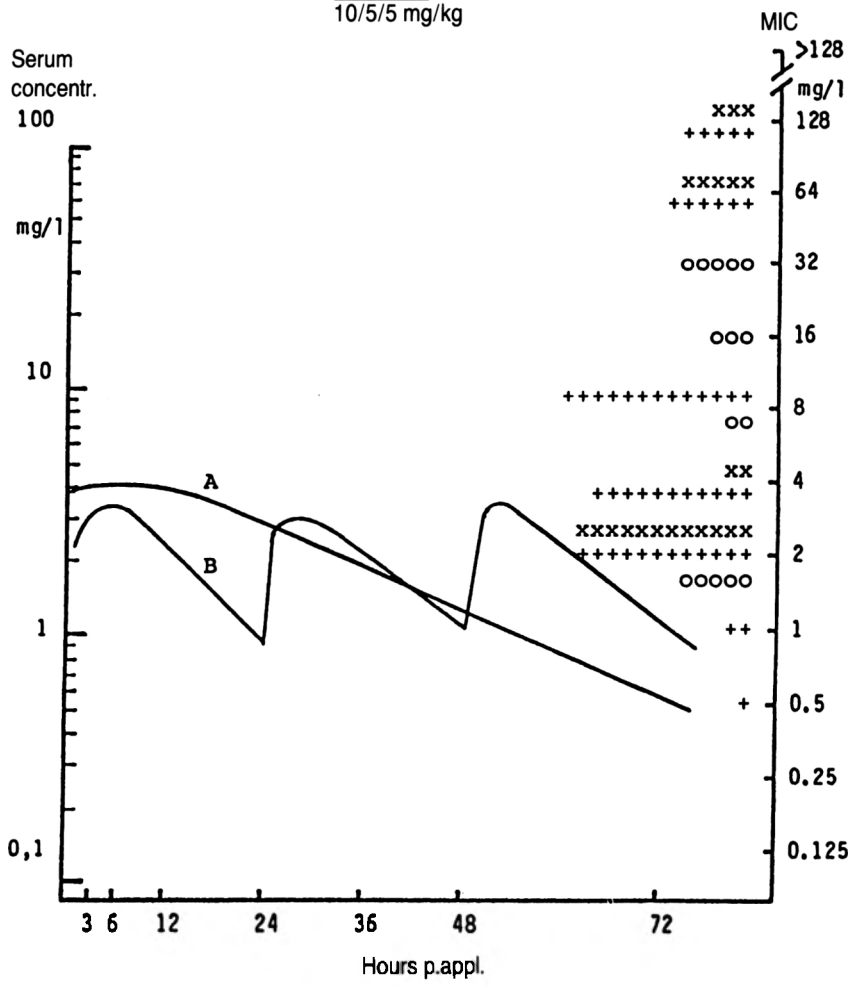


Figure 6: Tylosin concentration in the serum of healthy calves and cows after intramuscular, subutaneous and intravenous administration at increasing dosages (after HJERPE and ROUTEN, 1976; and ZIV and SULMAN, 1973) in relation to minimum inhibitory concentrations (MIC) for 72 strains of Pasteurella spp. and 15 strains of Corynebacterium pyogenes.

× = one strain of Pasteurella haemolytica
\+ = one strain of Pasteurella multocida
o = one strain of Corynebacterium pyogenes

Tylosin-Tartrate
A: 11 mg/kg
B,B': 20 mg/kg (bovine)
C,C': 55 mg/kg

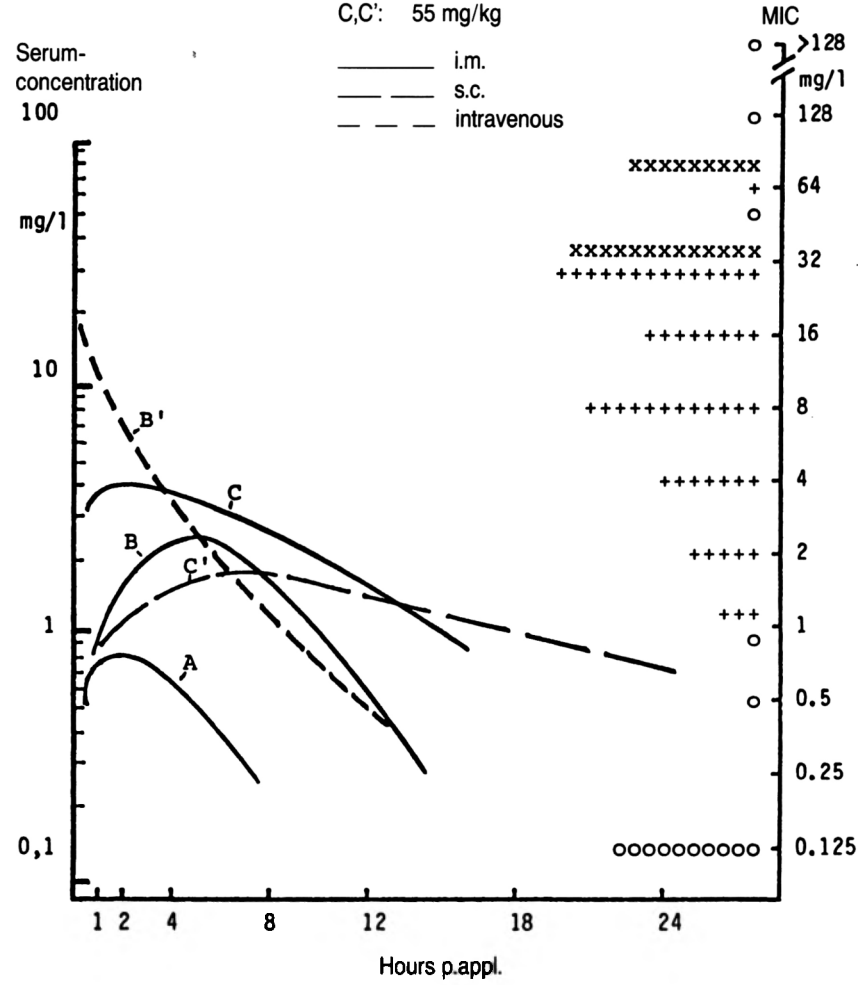


Figure 7: Spiramycin concentration in serum of healthy calves after intramuscular injection of a single dose of 50 mg/kg (after SCHIFFERLI et al., 1981 a) in relation to the minimum inhibitory concentration (MIC) for 72 strains of Pasterurella spp. and 15 strains of Corynebacterium.

× = one strain of Pasteurella haemolytica
\+ = one strain of Pasteurella multocida
o = one strain of Corynebacterium pyogenes

Spiramycin

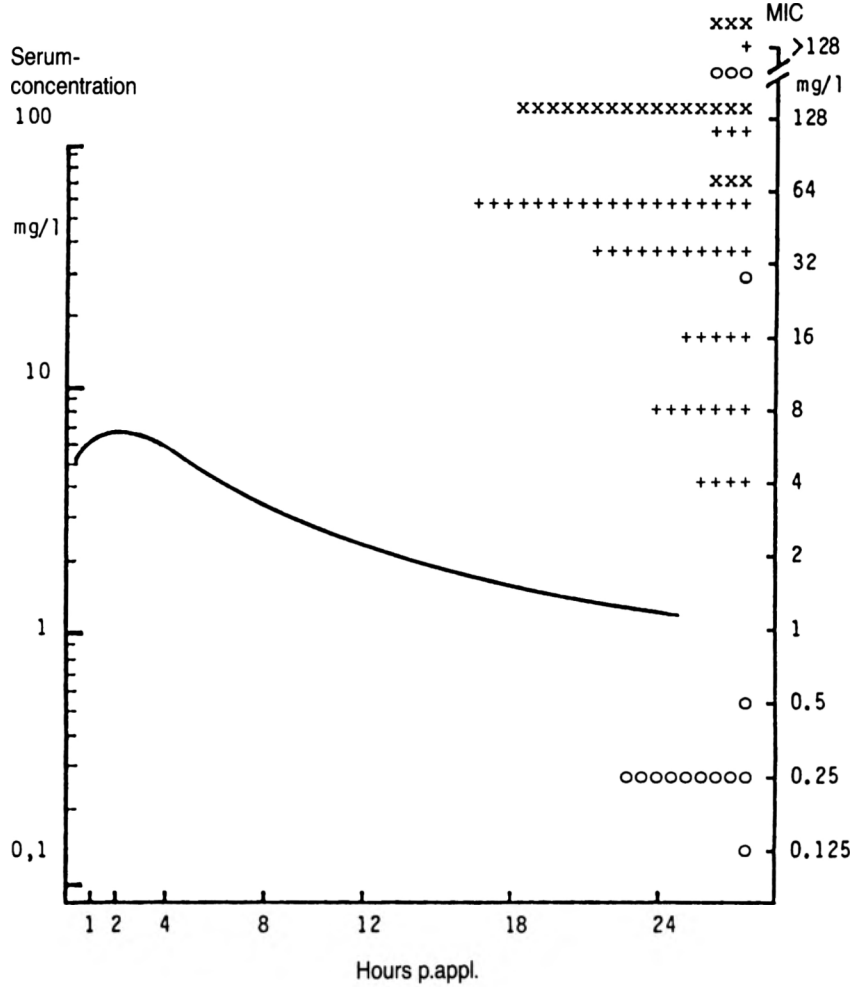
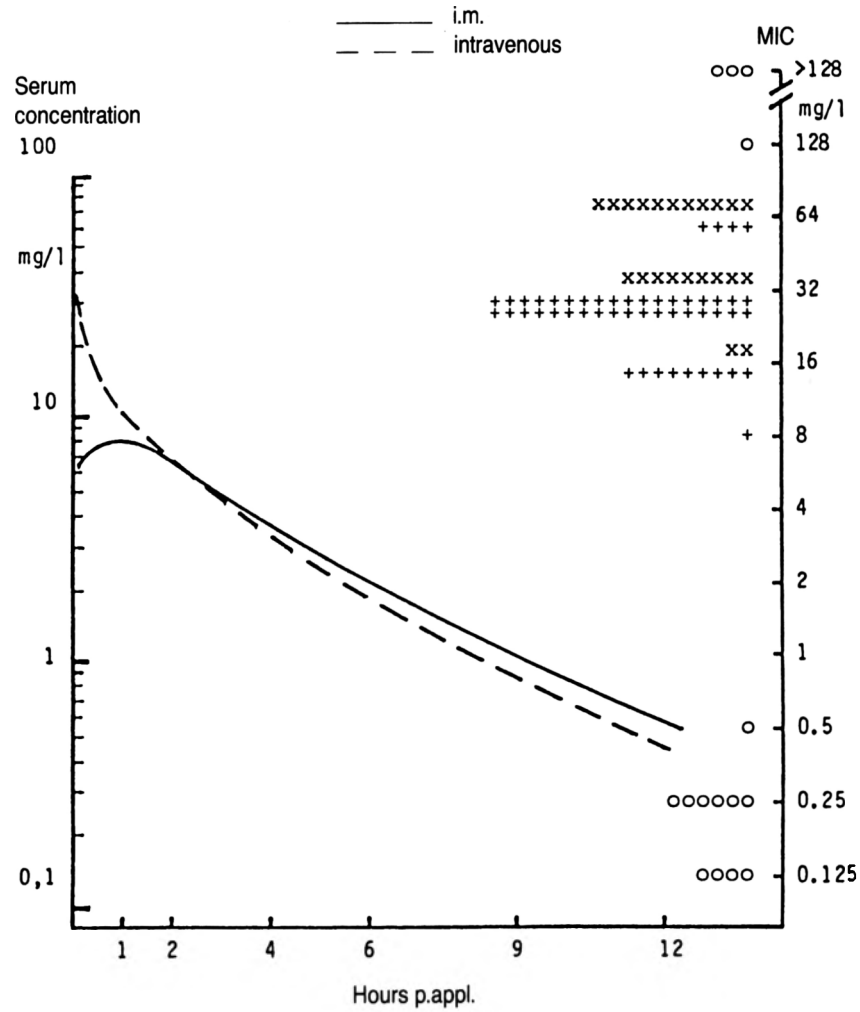


Figure 8: Lincomycin concentrations in serum of healthy calves after intramuscular or intravenous administration of a dose of 10 mg/kg (after BURROWS, 1980 a and 1983) in relation to minimum inhibitory concentrations (MIC) for 72 strains of Pasterurella spp. and 15 strains of Corynebacterium pyogenes.

× = one strain of Pasteurella haemolytica
\+ = one strain of Pasteurella multocida
o = one strain of Corynebacterium pyogenes

Lincomycin hydrochloride



respiratory tract of calves with enzootic bronchopneumonia (EBP) or from affected lung tissue of animals that had died, the sensitivity (respectively the resistance) was determined on the basis of minimum inhibitory concentration (MIC) to the following antibiotics: amoxycillin trihydrate, oxacillin sodium, neomycin sulphate, kanamycin sulphate, spectinomycin sulphate, tylosin tartrate, spiramycin, lincomycin hydrochloride, oxytetracycline hydrochloride and to a new chinoloncarbonic acid ester (only for C.p.). The results show that P. haemolytica and P. multocida proved to be very often sensitive to amoxycillin, often sensitive to kanamycin and only at relatively high concentrations sensitive to neomycin and spectinomycin; both germs were frequently resistant to oxacillin and oxytetracycline and to tylosin, spiramycin and lincomycin. C. pyogenes proved to be always sensitive to amoxycillin, oxacillin, kanamycin and spectinomycin, frequently sensitive to tylosin, spiramycin and lincomycin, frequently resistant to oxytetracycline and always resistant to neomycin. On the basis of the results, listed in two tables, the usefulness of the drugs for the treatment of EBP has been evaluated and discussed.

References

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