ANTIMICROBIAL SUSCEPTIBILITY OF *BRACHYSPIRA HYODYSENTERIAE* ISOLATES COMPARED WITH THE CLINICAL EFFECT OF TREATMENT

Vergelijking van antimicrobiële gevoeligheid van Brachyspira hyodysenteriae met het klinisch effect van behandeling

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ABSTRACT

The antibiotic resistance of *Brachyspira hyodysenteriae*, especially to pleuromutilins, is a matter of concern in several countries. In the present study, the antimicrobial susceptibilities of 30 Belgian *B. hyodysenteriae* isolates from 24 swine herds were tested and compared with the clinical effect of treatment. *In vitro,* no resistance to tiamulin was found, but two isolates (6%) were classified as intermediately susceptible. All isolates were susceptible to valnemulin at low concentrations (MIC50: 0.03 µg/ml). Higher minimal inhibitory concentrations (MICs) for valnemulin were found in isolates with higher MICs for tiamulin. For lincomycin, 16 (53%) isolates were classified as resistant and 4 (13%) isolates as susceptible. For tylosin, a high percentage of resistance (96%) was recorded. The MICs for 50% of the strains for salinomycin and doxycycline were 0.5 and 4 µg/ml, respectively.

Subsequently, the *in vitro* data obtained were compared with the farm history and clinical efficacies in 23 of the 24 swine herds of origin as judged by the attending veterinarians. The effect of treatment as evaluated in the field was generally in agreement with the *in vitro* data for these antibiotics. However, a clinical interpretation of certain breakpoints is imperative. A revision of the clinical breakpoint for tiamulin is proposed. Isolates with MIC 1µg/ml should be considered as not responding to therapy *in vivo*. Consequently, the therapeutic use of another compound is indicated.

In the third part of this study, the *in vitro* MIC for lincomycin was compared in detail with the effect of treatment on four farms. Even though *in vitro* all isolates were classified as resistant, a good response to treatment was observed on two farms. On one of these farms, however, the disease reappeared after treatment was discontinued. It was concluded that *in vitro* susceptibility testing of *B. hyodysenteriae* for lincomycin only partially predicted the clinical effect of treatment in the field.

SAMENVATTING

De antibioticumresistentie van *Brachyspira hyodysenteriae*, vooral tegen pleuromutilinen, stijgt in verschillende landen. De gevoeligheid van 30 Belgische *B. Hyodysenteriae*-isolaten uit 24 varkensbedrijven werd in deze studie onderzocht en vergeleken met het klinisch effect van behandeling.

In vitro kon geen resistentie aangetoond worden tegen tiamuline maar toch werden twee isolaten (6 %) als intermediair gevoelig geklasseerd. Alle isolaten waren gevoelig voor lagere concentraties valnemuline (MIC50: 0,03 µg/ml). Hogere minimaal inhibitorische concentratie (MICs) waarden voor valnemuline werden aangetroffen bij isolaten met hogere MICs voor tiamuline. Voor lincomycine werden 16 isolaten (53 %) geklasseerd als resistent en 4 (13 %) als gevoelig. Voor tylosine was een hoge graad van resistentie aanwezig (96 %). MIC50 voor salinomycine en doxycycline was respectievelijk 0,5 en 4 µg/ml.

Deze *in vitro* gegevens werden vergeleken met de bedrijfsdata en klinische gegevens verkregen van de bedrijfsdierenartsen voor 23 van de 24 bedrijven. Het effect van een behandeling was grotendeels in overeenstemming met de *in vitro* gemeten gevoeligheid. Desondanks is een klinische interpretatie van sommige breekpunten noodzakelijk. Om die reden wordt een herziening van het klinisch breekpunt voor tiamuline voorgesteld. Isolaten met MIC 1µg/ml dienen beschouwd te worden als niet reagerend op een behandeling waarbij een behandeling met een andere molecule aangewezen is.

In het derde deel van deze studie werd de *in vitro* MIC voor lincomycine in detail vergeleken met het klinisch effect van een behandeling op vier bedrijven. Hoewel *in vitro* alle isolaten resistent waren, werd op twee bedrijven een goede respons op de behandeling waargenomen. Bij één van die bedrijven echter dook de aandoening terug op na het stopzetten van de behandeling. Er werd geconcludeerd dat de *in vitro* gevoeligheidsresultaten van lincomycine slechts gedeeltelijk het klinisch effect van de behandeling in de praktijk kunnen voorspellen.

INTRODUCTION

Swine dysentery, a mucohemorrhagic colitis in pigs caused by the spirochete *Brachyspira hyodysenteriae*, was first described in 1921 and currently occurs in most swine producing countries. In Belgium, the prevalence of swine dysentery has increased in recent years. Not only did the number of submissions to the diagnostic laboratory in which the present investigation was carried out rise from 441 in 1999 to 868 in 2004, but also the number of positive samples in that period rose from 68 (15.4%) to 178 (20.5%) (unpublished results, Animal Health Care Flanders, 2004). Carrier animals, gilts as well as piglets, and fecal material are the most important sources of transmission of the disease in Belgian swine herds.

A limited number of antimicrobial agents are available for the treatment of swine dysentery. The pleuromutilins, tiamulin and valnemulin, are the antibiotics of choice and are also used in elimination protocols (Lobová *et al.*, 2004). In several countries, resistance of *B. hyodysenteriae* against several antibiotics has been reported (Gresham *et al.*, 1998; Lobová *et al.*, 2004). In Belgium, susceptibility testing of *B. hyodysenteriae* dates from 1997 (Hommez *et al.*, 1998a). In vitro resistance to lincomycin and especially to tylosin was substantial at that time, but no resistance was found against tiamulin.

In this study, the susceptibilities of recent Belgian *B. hyodysenteriae* isolates were investigated by determining the minimum inhibitory concentration (MIC) using the agar dilution technique. Most studies reporting *in vitro* susceptibility of *B. hyodysenteriae* give no additional information on the clinical effect of treatment. For veterinary practitioners, however, the response to treatment is of major concern. Therefore, on 24 farms an attempt was made to compare the *in vitro* data with field and clinical data as reported by the herd veterinarians.

To lincomycin, widespread resistance of *B. hyodysenteriae* has been documented (Buller and Hampson, 1994; Hommez *et al.*, 1998a; Molnár, 1996). The basis of this resistance was characterized as a mutation in the 23S rDNA (Karlsson *et al.*, 1999). Discrepancies between *in vitro* MIC and clinical response on treatment with lincomycin have been reported (Smith, 1990). In the final part of the present study the effect of lincomycin treatment on four farms with clinical dysentery was monitored and compared to the *in vitro* susceptibilities of the isolated strains.

MATERIALS AND METHODS

Sample collection, spirochete culture and identification

Samples were submitted to the laboratory for diagnosis of swine digestive disorders. Feces or colon scrapings were cultured on modified Trypticase Soy Agar (TSJ-BJ) agar with 5% sheep blood and incubated at 42°C for three days in anaerobic conditions (Anaerogen, Oxoid). After purification, *B. hyodysenteriae* identification was based on hemolysis and biochemical testing, as described by Hommez *et al.*. (1998b). Thirty *B. hyodysenteriae* isolates were selected at random and originated from 24 farms distributed all over the country. They were isolated between July and October 2003, and kept at –80°C until susceptibility testing. The other tests requested for these samples were aerobic culture (n=10) for *Escherichia coli* and *Salmonella* species and anaerobic culture (n=2) to detect *Clostridium perfringens*.

Susceptibility testing

The following antibiotics were tested by means of an agar dilution method: tiamulin hydrogen fumarate (VMD, Belgium), valnemulin hydrochloride (Novartis, Switzerland), lincomycin hydrochloride (Pfizer, Belgium), salinomycin sodium (Intervet, Germany), tylosin base (Elanco Animal Health, The Netherlands), and doxycycline hyclate (Virbac, France). Plates with the appropriate amounts of antibiotic were prepared as described previously (Hommez *et al.* 1998a). The tested concentrations ranged in two-fold dilutions from 0.03 µg/ml to 8 µg/ml for tiamulin and valnemulin, from 2 µg/ml to 128 µg/ml for lincomycin, from 1 µg/ml to 128 µg/ml for tylosin, from 0.03 to 3 µg/ml for salinomycin and from 0.125 µg/ml to 128 µg/ml for doxycycline. Inocula with an optical density equivalent to 1 McFarland were prepared in sterile saline using a photometer (Vitek ATB 1550, BioMérieux, Brussels, Belgium). Bacterial suspensions were inoculated on the plates using a Denley multipoint inoculator (Mast) and incubated for 3 days in anaerobic conditions at 37°C. The MIC was recorded as the lowest concentration at which no distinct hemolysis was seen in the inoculum spot in comparison with the hemolytic effect on the antibiotic free control plates. Additionally, the reference strain *B. hyodysenteriae* B78 ATCC27164^T and an internal control strain of *B. hyodysenteriae* DC185 intermediately resistant to tiamulin (Hommez *et al.* 1998a) were also tested.

Clinical data

A questionnaire was sent to the herd veterinarians of the 24 farms with questions on general herd information and applied therapy of swine dysentery in detail (antibiotic, dose, duration of therapy), as well as the clinical response to the applied antimicrobial therapy. The clinical effect was classified as 'good' when symptoms disappeared completely after treatment, as 'doubtful' when symptoms were clearly reduced but not absent, and as 'poor / ineffective' when there was no or only minimal reduction of symptoms after the initiation of the therapy. The kinds of antibiotics used for treatment of other diseases during the previous six months, preventive antimicrobial treatments at weaning or at the start of the fattening period, and the use of antimicrobial growth promoters were also recorded.

Lincomycin field trial

Three fattening farms with 1000 (A), 3750 (B) and 1060 (C) pigs, respectively, and one mixed farm (D) with 250 sows and 1570 fatteners, all suffering an outbreak of swine dysentery, were incorporated in the last part of the study. On these four farms, clinical signs were compared with the *in vitro* susceptibility of the strain isolated. Evaluation of clinical signs on individual pigs was performed before treatment, one week after the start of treatment and at the end of the treatment period, always by the same person. Feces were scored 1 normal, 2 pasty, 3 liquid or 4 watery (very liquid, appearing as water dripping from the perineum), and the presence of blood and mucus was noted. The number of pigs was recorded, as well as the number of pens with clinical symptoms. The pigs were treated in feed with lincomycin (110ppm, Lincomix[R] 110, Pfizer) 5mg/kg/d body weight for 21 days. On farm D, treatment was adjusted after 7 days due to poor clinical effect by increasing the dose of lincomycin to 8mg/kg/d (5mg/kg/d in feed, 3mg/kg/d in water) and adding 6mg/kg/d spectinomycin (Linco-Spectin 100, Pfizer) to the drinking water.

Fecal samples were collected for culture and susceptibility testing for lincomycin of *B. hyodysenteriae* when clinical symptoms were present: in all cases before treatment and on two farms during treatment. Bacterial culture and susceptibility testing were performed as described above.

RESULTS

Laboratory examinations

In addition to the isolation of *B. hyodysenteriae* in all the selected samples, other intestinal pathogens were also isolated: *Escherichia coli* (n=8), hemolytic *E. coli* (n=1), *Salmonella* serotype Derby (n=2) and *Clostridium perfringens* (n=1).

Susceptibility testing

The results of susceptibility testing of 30 *B. hyodysenteriae* isolates are summarized in Table 1. On seven farms, the tested isolate originated from a sample taken during the chronic phase of the disease. From six farms, two isolates from different animals were tested. On one of these farms the tylosin and doxycycline MIC differed for more than one two-fold dilution between the two isolates, on the second the same was seen with tiamulin and lincomycin, and on the third farm with tiamulin and valnemulin. In this last case the isolates were obtained from samples taken on two different occasions

For tiamulin, the highest MIC recorded was 2 µg/ml. The MIC values for valnemulin were above the minimum concentration for only 5 out of 30 isolates tested. These MICs corresponded with higher MICs for tiamulin (Table 2). For lincomycin and tylosin, the MIC was mostly at the higher limit of the dilution range. Salinomycin showed MIC values within a narrow range, while MICs for doxycycline were mostly 2 or 4 µg/ml.

Clinical data

Data were collected from 23 out of 24 farms (response rate 95.8%): 10 breeding-fattening farms with a mean of 151 sows (range 30 - 300) and 891 fatteners (range 250 - 2300), and 13 fattening farms with a mean of 1226 pigs (range 20 - 4500). Oral medication was most common: tiamulin, lincomycin and tylosin were administered in feed or in water, valnemulin only in feed. Lincomycin was used parenterally in two cases. Ten farms used more than one antibiotic to treat dysentery – either a different drug for different categories of pigs or as successive treatments when the first drug had insufficient clinical effect. The therapeutic antibiotics used on these farms, their clinical effect as reported by the attending veterinarians and the MIC of the isolated strains are shown in Table 3. In most cases the MIC value was in accordance with the clinical effect on treatment: low MIC coincided with good clinical effect and higher MIC values with doubtful or no effect. One isolate with an MIC of 0.06 µg/ml for tiamulin was obtained from an outbreak that did not respond well to treatment. Clinical effect was seen with tylosin, although the isolate representing the farm had a high MIC for tylosin (>128 µg/ml). The percentage of isolates with MIC MIC50 did not differ for any antibiotic between farms with no use or no therapeutic use of an antibiotic and farms with prolonged use (chronic dysentery problem) or routine use of an antibiotic for other indications (e.g. doxycycline for respiratory problems).

Table 1. Antibiotic concentrations at which growth of 50% and 90% of 30 *B. hyodysenteriae* strains tested is inhibited (MIC50 and MIC90 in µg/ml) and MICs of the reference strains B78, DC185.

Antibiotic	MIC50	MIC90	Range	B78	DC185	Sensitive*		Resistant*	
						Breakpoint*	Isolates	Breakpoint*	Isolates
Tiamulin	0.125	0.5	0.03 - 2	<0.03	2		28	>4	0
Valnemulin		0.125		<0.03	1				
Lincomycin	64	128		<2	>128		4	>36	16
Tylosin	>128	>128	4 - >128	8	>128			>4	29
Salinomycin	0.5	0.5	0.06 - 0.5	0.25	8		0		
Doxycycline	4	8		0.5	8				

* MIC breakpoints (µg/ml) according to Rønne and Scanzer, 1990.

Table 2. Minimal inhibitory concentration (MIC in µg/ml) of valnemulin for *B. hyodysenteriae* strains above the minimum concentration tested (0.03µg/ml) and for the reference strain DC185, compared with their MIC for tiamulin.

Strain designation	Tiamulin	Valnemulin
14	0.25	0.06
15	0.5	0.125
28	1	0.25
43	2	0.25
46	2	0.25
DC185	2	1

The following drugs were used in the affected category for other indications or as routine treatments: doxycycline (n= 13), salinomycin (n=2), paromomycin (n=2), apramycin (n=1), colistin (n=4), sulfonamide-trimetoprim (n=5), amoxicillin (n=4) and florfenicol (n=1).

Lincomycin field trial

Farm A: Diarrhea (score 2 –3) without blood was observed in five pigs in three of 24 pens with pigs of about 40 kg before treatment. One week after treatment, the diarrhea had disappeared except in one pig (removed from its pen due to excessive fighting) with a score of 4 for diarrhea. At the end of the treatment, there was no more diarrhea and the pigs were more homogeneous in terms of body condition. There was no relapse during the rest of the fattening period.

Farm B: Two compartments were treated in the trial (pigs of about 105 kg). In compartment 1, five pigs in three pens (out of a total of 12 pens) showed diarrhea, and all had a feces score of 3 with blood staining. In compartment 2, eight pigs in five pens out of a total of 12 pens had clinical symptoms: five pigs showed a score of 3 with blood staining, while three pigs were given a score of 4 with blood staining. After one week and until the end of treatment no diarrhea was seen. However, the owner mentioned temporary diarrhea after partial removal for slaughter with accompanying food withdrawal and mixing of small groups. When the medication was stopped, the symptoms returned within a few days.

Farm C: Before treatment, nine pigs of about 50 kg in four of 30 pens were affected (seven with diarrhea score 3, and two with score 4). There was no blood staining and generally the pigs had a sunken appearance. One week after treatment, the symptoms were unchanged. *B. hyodysenteriae* was isolated from the feces. At the end of the 3-week treatment period, two pens had diarrhea: a score of 2 was noted once in two pigs, and a score of 4 was noted once in another pig, all without blood staining.

Farm D: Average weight of pigs was ca. 25 kg on this farm. Before treatment, at least one pig (in all but three of 16 pens had watery diarrhea. Half of the pigs had a score of 3, the other half a score of 4. These pigs had an unthrifty appearance and a large abdominal volume. One week after treatment, the symptoms were the same and *B. hyodysenteriae* was isolated from the feces again. The medication dose was increased to 8 mg/kg and spectinomycin was added as well, but at the end of the treatment period the clinical signs had hardly decreased at all. From one

Table 3. Therapeutic antimicrobial agents with their clinical effects as interpreted by the veterinarian in relation to the MIC (µg/ml) for the isolated strains (Total number of farms: n = 23; total number of strains tested from these farms: n = 29). MIC breakpoints for susceptibility: tiamulin and tylosin   1µg/ml, lincomycin   4 µg/ml (Rønne and Scanzer, 1990).

Antibiotic	Farm	Good effect		Doubtful or no effect	
	n	Farms (%)	Susceptible strains (%)	Farms (%)	Susceptible strains (%)
Tiamulin	17	15 (88)	15 (100)	2 (12)	1 (50)
Valnemulin	3	3 (100)			
Tylosin	5	1 (20)	0 (0)	4 (80)	0 (0)
Lincomycin	9	5 (55)**	3 (60)*	4 (45)	0 (0)
Lincomycin (field trial)	4	1 (25)	0 (0)	3 (75)*	0 (0)

* 1 farm with concurrent salmonellosis;
** 2 farms with concurrent salmonellosis.

fecal sample, *Salmonella* was isolated both before and during treatment.

The MIC for lincomycin of the isolates in this field trial was 64µg/ml on farm A and >128µg/ml on farm B. On farm C it was >128µg/ml both before and during treatment while on farm D the MIC was >128 and 128 µg/ml before and during treatment respectively.

DISCUSSION

The worldwide abundance of reported resistance or lowered susceptibility of *B. hyodysenteriae* to tiamulin and valnemulin (Buller and Hampson, 1994; Gresham *et al.*, 1998; Lobová *et al.*, 2004) necessitates regular MIC monitoring at the regional level.

In this study, the MIC50 for tiamulin was one dilution higher compared to the isolates tested in 1997 (Hommez *et al.*, 1998a). A more significant increase in the MIC50 for tiamulin was reported in other countries for the same period (Lobová *et al.*, 2004), which indicates that there was a minor increase in the antibiotic resistance of Belgian isolates. According to the breakpoints proposed by Rønne and Scanzer (1990) on the basis of the pharmacokinetic properties and tissue concentrations of the molecule, no resistance was found to tiamulin in this study. Following this criterion, two isolates were intermediately susceptible to tiamulin (>1 -   4 µg/ml). For one of these isolates for which we received clinical data, the reaction to therapy was considered insufficient. The herd veterinarian, unaware of the susceptibility of the isolate, blamed it on inadequate mixing of the antibiotic in the feed. Poor clinical response to tiamulin in Belgian isolates classified as intermediately susceptible was described in the reference strain DC185 (Hommez *et al.*, 1998a) and was seen in two recently tested isolates (Vyt, unpublished).

The field observations in this study confirm the point of view of Karlsson *et al.* (2003), who stated that the breakpoint of 4 µg/ml (Rønne and Scanzer, 1990) is too high to indicate decreased susceptibility, and therefore proposed a lower microbiological breakpoint for tiamulin of 0.5µg/ml. In order to obtain a clear indication of the clinical efficacy of tiamulin from the MIC, the results in this study also favor a revision of the clinical breakpoint proposed by Rønne and Scanzer**.** On the basis of the field data in this study and in accordance with statements in previous studies (Karlsson *et al.*, 2003; Lobová *et al.*, 2004), a clinical breakpoint for tiamulin of 1 µg/ml is proposed. This clinical breakpoint (1 µg/ml) can be used as a rule of thumb if adequate dosing of the antibiotic is ensured: isolates of *B. hyodysenteriae* with MIC lower than 1 µg/ml can be considered as responding to therapy, while the effects of similar treatments in outbreaks with MIC = 1 µg/ml isolates can be supposed to be doubtful or without *in vivo* effect. In this study, tiamulin isolates showing MIC of 0.5 µg/ml (n=3) and 1 µg/ml (n=1), as determined by agar dilution, were reported to be clinically effective. Nevertheless, since MIC determination by broth dilution renders results one two-fold dilution lower (Karlsson *et al.* 2003; Rhode *et al.*, 2004), and since one two-fold dilution is considered a tolerable variation between MIC tests (Råsbäck *et al.*, 2005), this clinical breakpoint for tiamulin of 1 µg/ml is universally applicable. Adjustment of the MIC breakpoint renders it not only more relevant for treatment of swine dysentery but is equally important when extrapolated to *Brachyspira* isolates of other species (Hampson *et al.*, 2006).

The MIC range for valnemulin was narrower compared to other studies with less high MIC values (Aitkin *et al.*, 1999; Rodhe *et al.*, 2004). In this study, individual isolates with high MIC for valnemulin also had high MIC for tiamulin. Since the susceptibility of *B. hyodysenteriae* to pleuromutilins is decreasing over time, a fact which parallels the increased use of pleuromutilins (Lobová *et al.*, 2004; Rodhe *et al.*, 2004), and since the MIC increase can be induced *in vitro* (Karlsson *et al.*, 2001), the use of pleuromutilins should be restricted to clear indications in order to prevent the development of resistance in *B. hyodysenteriae*.

The MICs for lincomycin, salinomycin and tylosin were in the same range as for the isolates investigated in 1997 (Hommez *et al.*, 1998a). For lincomycin, only 4 isolates (13%) were classified as susceptible (Rønne and Scanzer, 1990). Nevertheless, lincomycin is frequently used as a therapeutic agent in swine dysentery for its short withdrawal time and since in the past it could be used when salinomycin was present. In this study, there was no straightforward agreement between the *in vitro* MIC and the reported clinical effect for lincomycin. This lack of agreement may be due to concurrent intestinal disease such as salmonellosis, which is known to compromise therapy (Gresham *et al.*, 1998). However, in this study, on two farms with concurrent isolation of *Salmonella* Derby from the same sample, the response to lincomycin was reported as good. The involvement of other pathogens was not examined in every case because the examinations of the fecal sample were based on the practitioner's clinical diagnosis.

In the field trial, lincomycin was capable of reducing clinical symptoms on one farm even though the *in vitro* MIC of the herd isolate was classified as resistant. The clinical effect of lincomycin on isolates resistant *in vitro* was reported earlier (Smith, 1990) at a dose of 10mg/kg/d. Although a dose effect had been described previously (Hamdy and Kratzer, 1981), the dose was kept at 5mg/kg to respect withdrawal times. The involvement of other enteric pathogens (e.g. *Salmonella*, a species against which lincomycin is not active) may also play a role, as well as the effect of the molecule on other anaerobic bacteria in the gut (Buller and Hampson, 1994). The clinical symptoms of *B. hyodysenteriae* are indeed associated with the presence of other anaerobes in the gut (Robinson *et al.*, 1984; Whipp *et al.*, 1979). This effect on the intestinal flora might explain the quick relapse in cases of anorexia or when the medication was stopped. Severe gut lesions with survival of spirochetes in the colon wall may also explain the relapse of clinical symptoms after completion of the lincomycin treatment.

In conclusion, the correlation of MIC with field data in this study provided no indications of the induction of higher MIC values on chronically affected farms or on farms that routinely use the antimicrobials tested. For tiamulin, the use of the proposed new clinical breakpoint coincides better with the *in vivo* effect of the molecule as reported in this study.

ACKNOWLEDGEMENTS

To authors wish to thank P. Vanclooster for her excellent technical assistance, and the herd veterinarians for their follow-up in the field. They also want to express their appreciation to Novartis (Switzerland), VMD (Belgium), Pfizer (Belgium), Intervet (Germany), Elanco (The Netherlands) and Virbac (France) for providing the antibiotics, and to W. Neirynck (Pfizer) and G. Letessier (Virbac) for providing financial support for this study.

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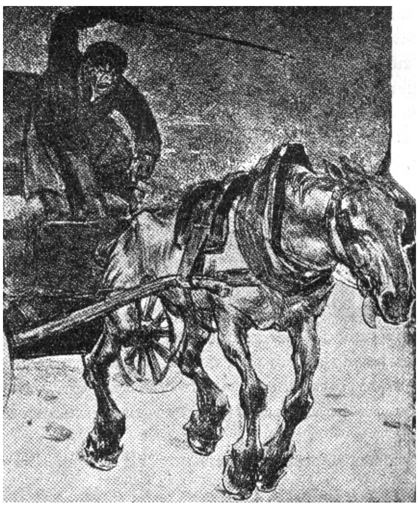
DE ZWEEP



Een der grootste misbruiken is wel de zweep.

In België kan men den kleinsten wagen niet ontmoeten, zonder een kilometerlange zweep te zien in de handen van den geleider. Het kind, dat "paardje" speelt, verlangt een zweep. Eerst dient zij voor het kartonnen paard; later deelen hond of kat ervan mede, en zoo krijgen de kinderen, al spelende, de gewoonte de zweep te gebruiken!

Els eersten **raad** beveel ik dan ook aan **de kinderen geen zweep als geschenk te geven**. Tehuis zal de porceleinkas er haar voordeel bij vinden; op straat zullen de oogen der voorbijgangers er bij winnen en de relletjes en vechtpartijtjes tusschen kleine bengels dikwijls vermeden worden.

Maar vooral: de kleine zal geen hardvochtige gewoonten aannemen. Hoe wreed is immers die korte, snelle beweging die een koord of snoer striemend doet neerkomen op lijf, armen of beenen (...)

Uit: ***Beschaven – Civiliser. Almanak 1940****, uitgegeven door de Kon. Maatschappij tot Bescherming der Dieren (Oost-Vl.) en der Maatschappij voor dierenbescherming van Brugge.*