

**COMPARISON OF THERAPEUTIC EFFECTIVENESS
OF IMMUNOMODULATOR VETOKEHL SUB D4
(SANUM-KEHLBECK) AND AMPICILLIN
IN TREATMENT OF *BRONCHOPNEUMONIA
ENZOOTICA CATARRHALIS VITULORUM***

D. Czernomysy-Furowicz, A. J. Furowicz

*University of Agriculture, Department of Immunology and Microbiology,
Szczecin, Poland*

The purpose of the experiment was to compare the efficiency of treating clinical forms of bronchopneumonia with Vetokehl Sub D4 modulator, with ampicillin and immunomodulator with antibiotic. Bronchopneumonia is an infectious viral disease characterized by a deep immunosuppression, and it is complicated by bacterial infections (Markowska-Daniel et al., 1991). The assumption is that guided destruction of bacteria by the antibiotic and the stimulation of the immunity mechanisms (by Vetokehl Sub D4) should result in positive effects of the therapy.

MATERIAL AND METHODS

30 calves with the clinical symptoms of *bronchopneumonia catarrhalis enzootica* (acute forms), of the Lowland Black-white breed from the West Pomerania region were included in the examination. The animals were divided into the following experimental groups:

Group 1: 10 calves (weight of about 80 kg) were treated with ampicillin 20% (Bremer Pharma GMBH) in doses of 3-4 g per animal. The antibiotic was given intramuscularly, only once.

Group 2: 10 calves (weight about 80 kg) were immunostimulated subcutaneously with Vetokehl (*Bacillus subtilis* D4; Sanum Kehlbeck) 2x weekly à 1 ml (0 and 5 day) and treated with ampicillin. A single dose of the immunomodulator was 1 ml dilut. aquos of *B. subtilis* D4 (1 ampulle of Vetokehl Sub D4) per animal. The doses and way of administration of antibiotic were the same as those in group 1.

Group 3: 10 calves (weight about 80 kg) were stimulated with Vetokehl Sub D4. The doses and way of administration were identical with those in group 2.

Immunomodulator and antibiotic

The animals were immunized with the Vetokehl Sub D4 preparation Sanum-Kehlbeck, D-2812 Hoya (Vetokehl Sub D4-information, 1993) and treated with ampicillin (Ampicillin 20% Bremer Pharma GMBH).

Immunological tests

The cell immunity was determined by means of the following tests: the phagocytosis test (the Hamburger number and Wright index) according to Wright's method modified by Doležal, the nitroblue tetrazolium reduction test (the NBT test) as described by Park et al. and Czernomysy-Furowicz et al. (spectrofotometric method) (Czernomysy-Furowicz, Furowicz, 1991), the test of blast transformation of lymphocytes with LF-7 as described by Angus and Yang, the esterase test as described by Mueller and Koska. At the same time the hematocrit and the counts of peripheral erythrocytes and leucocytes, as well as leucocytic picture were determined. In order to determine humoral immunity blood serum was examined. Total protein in the blood serum was determined by the biuret method (as described by Gornell et al.), whereas the electrophoretic division was carried out on cellulose acetate (manufactured by Oxoid company in veronal buffer of pH = 8.6, ionic strength = 0.5). In the experiment the densitometer manufactured by the Kipp and Zonen company was used. The immunological tests were carried out on the basis of data obtained from materials by Outeridge (1985), Schmolke, Vorlaender (1983) and Šlopek (1970). For the esterase test some materials from the work by Mueller et al. (1975) were used. The two factors variance analysis and Duncan test were employed to verify the statistical hypothesis.

Clinical examination

Immunological monitoring (blood serum) had been carried out in all the groups before the therapy began and then it continued one and three weeks after the treatment had ended. For about eight weeks all the animals were under thorough clinical examination and the rate of growth (weighing) was checked every two weeks. The calves of all groups were kept in similar indoor conditions and fed the same fodder.

RESULTS

Clinical and zootechnic observations

The therapeutic effectiveness of both the immunostimulating treatment and antibiotic therapy linked with it, was noticed. In the calves from these groups regression of the disease symptoms was already seen a week after the beginning of the therapy (Tab. I). In the third week after the first administration of Vetokehl only two animals (1.0%) in these groups showed the symptoms of pneumonia and after six weeks they could be regarded as clinically healthy (Tab. I). The disease symptoms regressed a little faster in the groups of calves treated simultaneously with antibiotic and immunomodulator Vetokehl, whereas the disappearance of the disease symptoms was much slower in the ampicillin treated animals. Pneumonia, bronchitis, cough, fever and mucopurulent efflux were still observed in 55.6% of the calves in the third week after the beginning of the treatment and in 33.3% of the animals in the eighth week after this therapy (Tab. I). One calf of the analysed group died. Daily observations showed that the calves treated with Vetokehl grew most quickly (an average weight increase = 32.3 kg; a daily weight gain = 576.7 g). Animals treated with Vetokehl and ampicillin showed the following parameters: an average weight increase = 27.5 kg, a daily gain = 491.0 g. The slowest growth was observed in the calves under antibiotic therapy (parameters: 24.0 kg and 428.5 g, respectively) (Tab. II).

I. The occurrence of bronchopneumonia in the calves treated by different kinds of therapy (n = 10)

Method of therapy	Time from the first giving of drugs							
	1 week		3 weeks		6 weeks		8 weeks	
	clinical symptoms*	exitus	clinical symptoms*	exitus	clinical symptoms*	exitus	clinical symptoms*	exitus
Antibiotic therapy	6/10	1	5/9	0	4/9	0	3/9	0
Vetokehl** + ampicillin	4/10	0	1/10	0	0/10	0	0/10	0
Immunostimulation (Vetokehl**)	5/10	0	1/10	0	0/10	0	0/10	0

** modulator was given for the second time; * lesions in bronchia and lungs (bronchopneumonia), cough, mucopurulent rhinorrhea, fever; numerator - number of ill animals; denominator - number of animals in group

II. Average increase in weight of calves treatment with ampicillin and Vetokehl Sub D4; time of observation 56 days ($n = 10$)

Method of therapy	Average weight* (kg)		Average increase in weight (kg)	Average daily gain (g)
	initial weight	final weight		
Antibiotic therapy	78.0	102	24.0 ^a	428.5 ^a
Vetokehl + ampicillin	82.0	109.5	27.5	491.0
Immunostimulation	79.5	111.8	32.3 ^a	576.7 ^a

* measurement weight were made every two weeks

Immunological tests

In the animals stimulated with Vetokehl and in calves stimulated and simultaneously treated with ampicillin there was an increase in counts of peripheral leucocytes. However, the increase was not statistically significant as compared with the results in the group of calves treated merely with ampicillin.

Phagocytosis tests. A markedly increased percentage of polymorphonuclear leucocytes (PMNL) active in the process of phagocytosis (Hamburger number) was observed in the calves treated with Vetokehl combined with ampicillin a week after the immunization. In the calves merely immunized with Vetokehl an increase in the calves treated only with the antibiotic a decrease in percentage of PMNL was noticed after a week (Tab. III). Moreover, in the group of animals treated with Vetokehl along with antibiotic a significant increase in value of the Hamburger number was observed as compared with analogous markers in the group of calves merely immunized (Tab. IV).

The NBT test. In the ampicillin combined with Vetokehl treated calves showed a significant increase in percentage of PMNL reducing nitroblue tetrazolium (1 and 3 weeks after the beginning of the therapy). In the calves which were merely immunized with Vetokehl an increase in the value of this marker was observed a week after the immunomodulation (Tab. III). The increase in percentage of NBT positive granulocytes was also found out in the groups of animals treated with ampicillin along with Vetokehl and immunostimulated with Vetokehl was significant as compared with the number of these cells in the calves treated merely with ampicillin (Tab. IV).

The esterase test. In the groups of the animals treated with the antibiotic and modulator and merely immunized with Vetokehl a significant in percentage of esterase-positive lymphocytes was observed 1 and 3 weeks after ad-

III. The influence of ampicillin, Vetokehl Sub D4 and ampicillin or Vetokehl on polymorphonuclear leucocytes (PMNL) activity of calves with clinical symptoms of bronchopneumonia ($n = 10$)

Tests		Therapy	Time of observation					
			before stimulation		1 week after stimulation		3 weeks after stimulation	
			\bar{x}	s	\bar{x}	s	\bar{x}	s
Phagocytosis	Hamburger number	ampicillin	43.41	2.8	32.1	4.2	35.8	2.0
		Vetokehl + ampicillin	4.05 ^a	3.9	48.5	4.0	47.8	3.1
		Vetokehl	32.5 ^a	3.0	40.5	2.0	42.0 ^a	2.3
	Wright's index	ampicillin	2.7	0.6	1.6	0.5	1.9	0.3
		Vetokehl + ampicillin	1.5	0.5	2.4	0.6	3.6	0.4
		Vetokehl	1.7	0.7	1.8	0.5	2.9	0.5
% of NBT-positive cells	ampicillin	1.8	0.3	2.0	0.8	1.9	0.6	
	Vetokehl + ampicillin	1.0 ^{ab}	0.4	2.9 ^a	0.5	2.5 ^b	0.6	
	Vetokehl	1.2 ^a	0.4	2.3	0.3	2.9 ^a	0.7	

For Tabs. III-VIII:

n - number of calves in each group; \bar{x} - arithmetic mean; s - standard deviation; a, b - statistical difference, significant at $P < 0.05$

IV. The influence of ampicillin, Vetokehl Sub D4 and ampicillin or Vetokehl on polymorphonuclear leucocytes (PMNL) activity of calves with clinical symptoms of bronchopneumonia ($n = 10$)

Tests		Method of therapy					
		antibiotic therapy		Vetokehl + ampicillin		immuno-stimulation	
		\bar{x}	s	\bar{x}	s	\bar{x}	s
Phagocytosis	Hamburger number	38.8 ^a	3.0	45.6 ^{ab}	2.4	38.3 ^a	2.4
	Wright's index	2.1	0.5	2.5	0.5	2.1	0.6
% of NBT positive cells		1.9 ^{ab}	0.6	2.1 ^b	1.0	2.1 ^a	0.5

ministration of the preparations. In the calves treated with ampicillin a decrease in percentage of these lymphocytes was noticed after 3 weeks (Tab. V). It also found out that increase in percentage of esterase-positive lymphocytes in the calves treated with modulator and antibiotic as well as in

V. The profile of cellular immunity of calves with clinical symptoms of bronchopneumonia which were treated with Vetokehl Sub D4, Vetokehl and ampicillin or ampicillin ($n = 10$)

Tests	Therapy	Time of observation					
		before stimulation		1 week after stimulation		3 weeks after stimulation	
		\bar{x}	s	\bar{x}	s	\bar{x}	s
% of esterase-positive lymphocytes	ampicillin	59.6	2.9	59.1	2.4	58.7	1.3
	Vetokehl + ampicillin	61.0 ^{ab}	1.9	80.3 ^a	1.9	78.3 ^b	2.9
	Vetokehl	60.8 ^{ab}	2.3	71.3 ^a	4.0	73.3 ^b	3.1
Lymphoblastic transformation with LF-7 (%)	ampicillin	9.8	1.2	9.3	1.8	11.6	2.0
	Vetokehl + ampicillin	12.3 ^{ab}	0.8	24.2 ^a	2.1	20.0 ^b	1.4
	Vetokehl	11.1 ^{ab}	1.4	22.0 ^a	2.0	19.6 ^b	1.1

VI. The profile of cellular immunity of calves with clinical symptoms of bronchopneumonia which were treated with Vetokehl Sub D4, Vetokehl and ampicillin or ampicillin ($n = 10$)

Tests	Method of therapy					
	antibiotic therapy		Vetokehl + ampicillin		immuno-stimulation	
	\bar{x}	s	\bar{x}	s	\bar{x}	s
% of esterase positive lymphocytes	59.1 ^{ab}	2.2	73.2 ^a	2.2	68.5 ^b	3.1
Lymphoblastic transformation with LF-7 (%)	10.2 ^{ab}	1.6	18.8 ^a	1.4	17.6 ^b	1.5

the animals stimulated with Vetokehl was significant as compared with the number of these cells treated merely with ampicillin (Tab. VI).

The blast transformation test. In the groups of animals immunized with Vetokehl and treated with ampicillin combined with Vetokehl a significant increase in percentage of lymphocytes stimulated with LF-7 was observed 1 and 3 weeks after the administration of the preparation (Tab. V). It was also found out that increase in percentage of these lymphocytes in the calves treated with the modulator and antibiotic and only stimulated with Vetokehl was significant as compared with the number of lymphocytes in the animals treated merely with antibiotic (Tab. VI).

Total protein and electrophoretic division of blood serum. A significant increase in level of total protein in the groups of animals stimulated with Vetokehl and those treated with modulator and the antibiotic was observed after 3 weeks (Tab. VII). As regards gamma-globulin fraction the highest

VII. The influence of ampicillin, Vetokehl Sub D4 and ampicillin or Vetokehl on the level of serum proteins of calves with clinical symptoms of bronchopneumonia ($n = 10$)

Tests (g/l)	Therapy	Time of observation					
		before stimulation		1 week after stimulation		3 weeks after stimulation	
		\bar{x}	s	\bar{x}	s	\bar{x}	s
Total protein	ampicillin	38.5	1.1	38.16	1.1	41.5	0.6
	Vetokehl + ampicillin	36.6 ^a	1.1	40.5	0.9	43.4 ^a	1.5
	Vetokehl	40.0 ^a	2.1	43.3	2.2	45.0 ^a	1.5
Albumins	ampicillin	14.3	0.5	15.0	0.6	15.9	0.9
	Vetokehl + ampicillin	14.6	0.8	16.7	0.7	17.5	0.9
	Vetokehl	16.6	1.2	16.9	0.8	17.3	0.7
Alfa 2-globulins	ampicillin	6.1	0.9	6.7	0.9	7.5	0.6
	Vetokehl + ampicillin	4.7	0.6	5.4	0.7	5.6	0.6
	Vetokehl	6.9	1.1	6.8	1.2	6.9	0.6
Beta-globulins	ampicillin	5.5	1.1	3.5	1.1	4.9	1.0
	Vetokehl + ampicillin	9.4	1.0	5.5	0.7	5.7	0.9
	Vetokehl	5.3	1.9	6.8	1.1	5.9	1.3
Gamma-globulins	ampicillin	12.6	0.7	13.0	1.3	13.2	0.7
	Vetokehl + ampicillin	11.3	2.2	12.9	1.9	14.6	2.2
	Vetokehl	11.2	1.0	12.8	1.0	14.9	1.4

increase was observed in the Vetokehl-immunized animals but it was not statistically significant. The comparison of the level of beta-globulin fraction in three groups shows the highest parameters in the group of the animals treated with the modulator and antibiotic (Tab. VIII).

CONCLUSIONS

1. The treatment of calf bronchopneumonia (*Bronchopneumonia enzootica catarrhalis vitulorum*) with a properly chosen antibiotic and by the Vetokehl immunostimulation leads to a fast regression of the disease symptoms.
2. Good results can also be achieved by stimulating ill animals with Vetokehl without antibiotic therapy.
3. Both systems of the therapy increase some parameters of the cell immunity (phagocytosis, NBT-test, blast transformation and esterase-tests).

VIII. The influence of ampicillin, Vetokehl Sub D4 and ampicillin or Vetokehl on the level of serum proteins of calves with clinical symptoms of bronchopneumonia ($n = 10$)

Tests (g/l)	Method of therapy					
	antibiotic therapy		Vetokehl + ampicillin		immunostimulation	
	\bar{x}	s	\bar{x}	s	\bar{x}	s
Total proteins	39.4	0.9	40.2	1.2	42.8	1.9
Albumins	15.1	0.7	16.3	0.8	16.9	0.9
Alfa 2-globulins	6.8	0.8	5.0	0.6	6.9	1.0
Beta-globulins	4.6 ^{ab}	1.1	6.9 ^a	0.9	6.0 ^b	1.4
Gamma-globulins	12.9	0.9	12.9	2.1	13.0	1.1

- Better effects are achieved by the combined administration of the antibiotic and immunomodulator than by the application of Vetokehl alone. Thus, it may be assumed that there is a cooperation of the ampicillin and Vetokehl effects (protective synergism).
- Both systems of treatment give markedly gains in weight but daily observation showed that calves treated with Vetokehl grew most quickly.
- The therapy with the ampicillin alone is less effective and does not remove immunodeficiency.

References

- CZERNOMYSY-FUROWICZ, D. – FUROWICZ, A. J.: Porównanie testu redukcji blekitu nitrotetrazoliowego przez obojetne granulocyty owcze w metodach cytochemicznej i spektrofotometrycznej. *Med. Weter.*, 47, 1991: 227–228.
- MARKOWSKA-DANIEL, I. – PEJSAK, Z. – FUROWICZ, A. J. – CZERNOMYSY-FUROWICZ, D. – SZMIGIELSKI, S. – JELIASZEWICZ, J. – PULVERER, G.: Prophylactic and therapeutic application of *Propionibacterium avidum* KP-40 in swine and calves with Acute Enzootic Bronchopneumonia. *Dtsch. tierärztl. Wschr.*, 98, 1991: 384–387.
- MUELLER, J. – BRUN DEL RE, J. – BUERKI, H. – KELLER, H. U. – HESS, M. – COTTIER, H.: Nonspecific acid esterase activity: a criterion for differentiation of T and B lymphocytes in mouse lymph nodes. *Eur. J. Immunol.*, 5, 1975: 270–274.
- OUTTERIDGE, P. M.: *Veterinary Immunology*. Sydney, Academic Press 1985.
- SCHMOLKE, B. – VORLAENDER, K. O.: Standardtechniken zur Bestimmung zellulärer Immunoreaktionen. In: VORLAENDER, K. (Ed.): *Immunologie, Grundlagen-Klinik-Praxis*. Stuttgart–New York, G. Thieme Verlag 1983.
- ŚLOPEK, S.: *Immunologia praktyczna*. Warszawa, PWL 1970.
- VETOKEHL SUB D4-information, Sanum-Kehlbeck, D-2812 Hoya, 1993.

Received for publication on December 22, 1996

CZERMOMYSY-FUROWICZ, D. – FUROWICZ, A. J. (Zemědělská univerzita, katedra imunologie a mikrobiologie, Štětín, Polsko):

Porovnání léčebné účinnosti imunomodulátoru Vetokehl Sub D4 (Sanum-Kehlbeck) a ampicilinu při léčení bronchopneumonie (*Bronchopneumonia enzootica catarrhalis vitulorum*).

Scientia Agric. Bohem., 27, 1996 (1): 29–37.

Cílem pokusu bylo porovnat účinnost léčení klinických forem bronchopneumonie telat modulátorem Vetokehl Sub D4, ampicilinem a imunomodulátorem v kombinaci s antibiotiky.

Bronchopneumonie je infekční virové onemocnění charakterizované hlubokou imunosupresí. Obvykle bývá zkomplikována ještě infekcemi bakteriálního původu (Markowska-Daniel et al., 1991). Předpokládá se, že řízené ničení bakterií antibiotiky a stimulace imunitních mechanismů (Vetokehl Sub D4) by mohly mít příznivý vliv na terapii.

léčení bronchopneumonie; modulátor Vetokehl Sub D4; antibiotika; ampicilin

Contact Address:

Dr Danuta Czernomysy-Furowicz, University of Agriculture in Szczecin, Department of Immunology and Microbiology, Dra Judyma 24 Street, 71-466 Szczecin, Poland