Vet. Med. J., Giza 40, No. 1, 89-110 (1992)

STUDIES ON THE EFFECT OF SPECIFIC IMMUNOSTIMULANT ON THE IMMUNE RESPONSE OF CHICKENS

I. Evaluation of the Immunopotentials of Newcastle Disease Virus-Specific Transfer Factor (NDV-S-TF) in chicken.

BY

M.F. EL-KADY*; R. SOLIMAN**; MANAL AFIFY ALI*, I. REDA***AND ADEL KHALID.****

* Dept. of Poultary diseases, Fac. Vet. Med., Cairo Univ. Egypt.
** Dept. of Clinical Laboratory Sciences, College of Applied Medical Sciences, King Saud Univ.
*** Dept. of Microbiology and Immunology, Fac. Vet. Med., Cairo Univ. Egypt.
**** Dept. of Microbiology, Fac. Vet. Med. Banha Univ. Egypt.

Received: 17.11.1991.

INTRODUCTION

There are situations in veterinary medicine in which it is desirable to enhance the immune response. These include the potentiation of normal immune response in order to enhance protection or to overcome the immunosuppressive conditions.

As the use of many molecules regulating immune responses is an active area of research to increase the body defence to most serious diseases. Transfer factor is one of these molecules which proved to have important immuno regulatory potential.

Transfer factor (TF) is a dialyzable leukocyte extract (DLE) which is capable of transfering cellular

immunity from skin test positive donors to a skin test negative recipient (Lawrence, 1955). The administration of this factor was found to induce lymphocyte stimulation and production of mediators of cellular immunity (lymphokines) in the presence of the same antigen against which the donor of the TF has been sensitized (Klesius and Kirkpatrick, 1983).

The TF preparations are non-antigenic and non toxic molecules which have no direct effect on the humoral immunity (Klesius and Kirkpatrick, 1983). The apparent lack of antigenicity and pathogenicity and the immunizing capabilities render it as an appropriate immunotheraputic and immunoprophylactic tool.

In studies carried out to evaluate the protective value of transfer factor preparations, the results have been encouraging, whereas dramatic improvement in resistance against several microbial and parasitic agents had been reported (Liburd et al., 1972, Schulkind, et al., 1972, Catanzaro and Spitler, 1987 Richard, et al., 1978; Miksiewicz et al., 1981; Klesius, 1982; Kita et al., 1984 and Smith et al., 1982). Moreover, using transfer factor therapy significant clinical improvment had been reported in many viral infections including disseminated vaccinia, measles, congenital herpes simples, Zoster cytomegalo virus, influnza virus and hepatitis B virus (Olson and Drube, 1978, Amanullah et al., 1981; Rodae, et al., 1985; Mayer, et al., 1985 and Carey, et al., 1987).

The present study was designed to evaluate the protective value of Newcastle Disease Virus specific transfer factor (NDV-S-TF) in chickens using the challenge test. Also, the immunomodulatory effect of NDV-S-TF on the immun response of chickens to NDV-vaccine was investigated.

MATERIAL AND METHODS

1. Material

* **Chickens:** Two hundred and seventy five, one day old commercial (Hubbard) from a private company were used for this investigation.

They were kept under standard hygienic conditions, fed on standard ration and watered d Lib.

* **Newcastle disease virus (NDV) strains:**

a. **Vaccinal strains:**

- Lasota vaccinal (Batch No. 2080 A) produced by Intervet International (B.V. Boxmeer-Holland) with titre of 10^9. $^1EID_{50}$ was used for vacciantion of experimental chicks.

- Oil adjuvant emulsion vaccine of Newcastle disease (BAtch lote 09055) produced by Intervet International (Boxmeer-Holland) was used for immunization of chickens used in preparation of (NDV-S-TF) transfer factor.

b. **Challenge strain:**

A local velogenic viscerotropic strain of NDV isolated and identified by Sheble and Reda, (1976) with an initial titre of 10^6 EID_{50}, 0.2 ml was used.

* **Media:**

- Lymphocyte sepration medium (Sigma U.S.A.) was used for separation of mononuclear leucocyte from peripheral blood.

Studies on the effect of specific immunostimulant..

- RPMI 1640 tissue culture medium was used in the lymphocyte transformation test.

- Sterile foetal calf serum (Gibco Limited, U.K., Batch No. 310620 A).

*** Reagents:**

- Heparin solution ampules (5000 i.u.) were used as anticoagulant.

- Phytohaemagglutinin-P (PHA) Sigma, U.S.A. (Lot No. 25F-9640) used as a non-specific mitogen in the lymphocyte transformation test.

*** Stains:**

- Trypan blue stain (Difco Laboratories U.K.) was used for staining of lymphocytes to determine their viability.

- Acridin orange (Sigma U.S.A.) was used as 1% solution for counting lymphocytes.

*** Kits:**

Kits for glucose consumption (God. Pap method).

Enzyme kits for sugar consumption for detection of residual glucose in RPMI medium (Bohringer Manheim GmbH Diagnsotica, West Germany Cat. No. 986721) were used.

Methods:

1. Preparation of Newcastle disease virus specific transfer factor (NCV-S-TF).

a. Separation of lymphocytes:

Blood was collected from one hundred 6 weeks old chickens immunized with oil adjuvant NDV vaccine.

Three weeks post immunization, the vaccinated chickens were slaughtered and blood was collected in sterile vials containing preservative-free sodium heparin (20 i.u./ml) as anticoagulant. The collected blood was used as a source of mononuclear leukocytes (CMNC) for the preparation of Newcastle disease virusspecific transfer factor.

Separation of monouclear leukocytes (MNC): the mononuclear leukocytes were separated using density gradient centrifugation on ficoll-hypaque Sp. gr. 1.077 (Boyum, 1966). The harvested MNC were washed 3x with sterile PBS, pH 7.2 and the cells were finally resuspended in RPMI-1640 medium. The recovered cells from all blood samples were pooled and resuspended in RPME 1640 medium. Using trypan blue exclusion test (Huddson and Hay, 1980), they viability and count of the lymphocytes/ml were determined.

b. Preparation of lymphocyte dialysate (NDV-S-TF):

Lymphocytes cell suspension containing 4×10^{10} lymphocytes were subjected to 10 cycles of freezing and thawing at 40°C and 37°C respectively. This was followed by sonication for 15 min. Using Sonicator apparatus Dynatech Sonic Dismenbrator.

The lymphocyte cell lysates (200 ml) were placed in dialysis bags* and dialyzed against 10 volumes of sterile dist. water (4 liters) at 4°C for 96 hours. Then the dialysate was devided into aliquotes of 10 ml, lypholized and kept frozen until used.

c. Calculation of lymphocyte count giving transfer factor dose in 10 ml of the dialysates:

The calculation was performed according to the equation (Mostafa, 1991):

---

* Cellophane tubing were obtained from Union Carbide Coroporation 633 West Chicago, Illinois, U.S.A.

$$\text{No. of Lymphocytes giving TF in 1 ml dialysate} = \frac{\text{No. of lymphocytes in the dialysis bags.}}{\text{Volume of dialysing fluid}}$$

In the present work 10 ml of the dialysate content of one vial was adjusted to contain transfer factor extracted from 1 x 10^8 cells.

Sterility test on the prepared TF was made by cultivation of some randomly selected vials on routine bacteriological media according to Cruickshank,et al., (1985).

2. **Lymphocyte transformation test:**

A modified method of Lucy (1974 & 1977) Charles et al. (1987).

3. **The Blastogenic response of peripheral blood lymphocyts**

Was measured through biochemical estimation of residual glucose in culture media using the glucose consumption test described by Shimakura et al., 1985; Ishikawa and Shirahata, 1986 and Ramadan et al. (1991).

4. **Haemagglutination inhibition test:**

Was carried out according to Glanck (1979) and the antibody titre was expressed as Geometric mean titre (GMT) according to Brugh (1977).

EXPERIMENTAL DESIGN

As illustrated in Table 1, one hundred and seventy five 32 day old chicks were divided into seven chiken groups (A-D) consisted of 25 chicken each. These

chickens did not receive any vaccine or drug before the beginning of the experiment. Chickens in group A were kept as negative control group. They were neither vaccinated no treated with transfer factor therapy. Chickens in group B were vaccinated against NDV using Lasota NDV vaccine intra occularly. Chickens in groups C, D and E were treated both with Lasota NDV-vaccine and NDV-S-TF therapy, however, in a different manner, where group C recieved the vaccine and the NDV-S-TF simultaneously. Chickens in group D, however, received ND-S-TF therapy 3 days before NDV vaccination. The NDV-S-TF therapy was given by I/M injection in the two groups. In group E, however, TF was given orally and simultaneously with Lasota NDV-vaccine. Chicken in the remaining groups were treated only with transfer factor therapy which was given by I/M route in gorup F, and orally in group G.

Evaluation of the immunopotentials of the NDV-S-TF was done using challenge assay, measurment of the developed antibodies using haemagglutination inhibition test the antibody titer were expressed as Geometric mean titre (GMT) according to Brugh, (1977) and also, by measuring the extent of lymphocyte blastogenesis using the glucose consumption assay.

RESULTS

1. Effect of NDV-S-RF therapy on the extent of transformation and proliferation of chicken lymphocytes in response to phytohemagglutinin (PHA) and NDV antigen:

Data presented in **Table (2)** show the extent of blastogenesis of lymphocytes obtained from chckens differently treated with NDV-S-TF as measured by glucose consumption assay. Lymphocytes obtained from chickens treated with NDV-S-TF and NDV vaccine

Table (1):
Experimental design

Chicken group	No. of Chickens group	NDV vaccine*	NDV-S-TF**	
			I/M	Orally
A	25	-	-	- (negative control)
B	25	+	-	- (vaccine control)
C	25	+	+***	- Combined therapy
D	25	+	+****	- Combined therapy
E	25	+	-	+ Combined therapy
F	25	-	+	-
G	25	-	-	+

* NDV vaccine used was intraocular insitilating of LaSota NDV-vaccin.

** The dose given was 5 vials/chicks = Which means that each bird received dialysate obtained from 5 x 10^8 lymphocytes lysate.

*** Both the NDV-vaccine and the NDS-S-TF were given simultaneously.

**** NDV-S-TF was given 3 days before vaccination with NDV-vaccine.

(groups C, D and E) showed significantly higher stimulation indices compared with stim. index of lymphocytes from control groups (A & B). Again lymphocytes from chicken groups treated with NDV-S-TF alone (groups F and G) showed markedly higher stimualtion indices as compared to the negative control groups A and B. However, the immunopotentiating effect of the NDV-S-TF therapy alone was particulary strong when the lymphocyte transformation was measured 3 days after adminstration. Then, it rapidly decreased to the normal levels of unstimulated lymphocytes.

2. The effect of NDV-S-TF therapy on the humoral immune response developed against NDV-vaccine:

Results shown in **Table (3)** doeument the immunoptentiating effect of NDV-S-TF on humoral immune response developed against NDV-vaccine. The most strongest immunopotentiating effect was observed in chickens received the NDV-S-TF orally at the same time of the NDV-vacciantion where a geometric mean titres (GMT) of 142.9 and 55.0 were measured in sera of these chickens (group E) after one and two weeks post vaccination respectively. Compared with a GMT of 5.7 and 27.9 measured after the Same time intervals in chickens vaccinated with NDV-vaccine alone (group B). Again as compared with the control group B highly significant rise in GMT against NDV vaccine was observed in chicken simultaneously treated with NDV-S-TF (I/M) plus NDV-vaccine, where a GMT of 107.2 and 119.4 were measured after the same time intervals respectively.

Injection of NDV-S-TF,three days before vaccination with NDV vaccine stimulated relatively higher GMT which reached to 29.0 and 64.0 when measured at one and 2 weeks post vacciantion. However, the simultaneous adminstration of the NDV-S-TF with the DNV - vaccine was the most effective way.

Table (2):
Effect of NDV-S-TF administration of lymphocyte transformation measured b glucose consumption assay

Chicken groups	NDV-vaccination	NDV-S-TF		Stimulation index£					
				3 days post NDV-S-TF therapy		1 week post administration of NDV-S-TF therapy		Two weeks post administration of NDV-S-TF therapy	
		Treatment	Route	PHA	NDV	PHA	NDV	PHA	NDV
A	-	-	-	1.80	1.19	1.32	1.17	1.41	1.18
B	+	-	-	2.70	2.10	2.90	1.66	2.50	1.19
C	+	+	I/M*	4.50	4.20	4.10	3.70	3.10	2.01
D	+	+	I/M**	3.96	3.01	3.02	2.70	2.20	1.77
E	+	+	Oral*	4.36	3.98	4.18	3.70	2.80	1.29
F	-	+	I/M	3.92	3.01	2.10	2.06	1.51	1.30
G	-	+	Oral	3.60	2.90	1.90	1.30	1.20	1.06

£ Stimulation index.

* NDV-S-TF was given simultaneous with the NDV vaccine.

** NDV-S-TF was given 3 days before the NDV vaccine.

3. Effect of NDV-S-TF therapy alone or combined with NDV-vaccine on chicken resistance to challenge with virulent NDV strain:

As shown in **Table (2)** administration of NDV-S-TF together with NDV-vaccine (groups C,D and E) induced 100% protection rate, compared with 86.6% protection rate in chickens receiving the vaccine alone (group B). In groups treated with the NDV-S-TF alone, protection rates of 20% and 13.6% were recorded in chickens receiving the NDV-S-Tf I/M (group F) or orally (group G) respectively. Compared with a zero protection rate in the negative control chickens (gorup A).

DISCUSSION

The concept of immunopotentiation has become increasingly important in recent years, particularly in regard to enhancing responses against infectious agents. Newcastle disease virus is the most serious disease in Egypt which lead the research to enhance the immune response against it and improving the immune resposne of its vaccine specially by specific immunostimulant which potentiate the cell-mediated and humoral immune response to this virus.

Transfer factor (TF) is a dialyzable leukocyte extract which is capable of transfering specific cell mediated immunity (CMI) from immunized donors to negative recipient. It has a low molecular weight, is non antigenic and non toxic (Baram and Condoulis, 1970 and Burger and Wayburn, 1971). Nevertheless, the efficacy of TF to transfer cell mediated immunity is not influenced by the maternal immunity (Asher et al., 1978).

All the abovementioned properties of the TF nominate it as an ideal immunizing agent particularly against

Table(3):
Effect of NDV-S-TF factor adminstration on the geometric mean titres (GMT) of haemagglutination inhibition antibodies developed against NDV-vaccine.

Chicken group	NDV-vaccination	NDV-S-F		GMT@ of HI measured after	
		treatment	Route	1 week post vaccination	2 weeks post vaccination
A	-	-	-	0.00	0.00
B	+	-	-	5.70	27.90
C	+	+	I/M#	107.20**	119.40**
D	+	+	I/M##	29.90*	64.00*
E	+	+	Oral	142.90**	55.70*
F	-	+	I/M	0.50	0.70
G	-	+	Oral	0.50	0.70

@ The result are a mean avearage of 10 pooled samples from each group.

NDV-S-TF was given simultaneously with the NDV-vaccine.

NDV-S-TF was given 3days before the NDV-vaccine.

* Significant (P<0.05).

** Highly significant (P<0.01).

infections which are dependant on cell mediated immune responses like viral diseases (Lobugio and Neidhart, 1976 and Nikonenko et al., 1990).

In the present study by using glucose consumption assay the ability of NDV-S-TF to transfer CMI was investigated. The obtained results **(Table, 2)** document clearly this capability where the lymphocyte blastogenesis response was markedly potentiated in NDV-S-TF treated chickens not only against the PHA polyclonal T-cell mitogen, but also against the NDV antigens. This indicate the specific transfer of cellular immunity and also general potentiation of the lymphocyte response (non-specific activation). The efficacy of TF in transfering specific CMI and non specific potentiation of lymphocyte has already been reported by many investigators (Shusuki Tsuji et al., 1964; Liburd, et al., 1972; Gallin adn Kirkpatruck, 1974; Fundenberg et al., 1980; Klesuis et al., 1980; Ashorn, et al., 1983; Georgescu et al., 1985; Joulian, et al., 1985; Mayer, et al., 1985 and Nikonenko., et al., 1990).

Also it was proved that NDV-S-TF was effective not only parentrally but also by oral route and in some experiments (see **Table, 4**). This route was even more effective than the other routes. The administration route independancy of the TF preparation has been reported by several authors. (Ascher et al., 1978; Ross and Halliday, 1982 adn Klesius and Kirckpatrick, 1983). This extra advantageous property of the TF as immunizaing agent is of outmost importance, particularly in massive vaccination programms.

In concern with the effect of TF on the humoral immune response, it has been reported that TF does not induce direct stimulation of specific humoral immunity (Khan et al., 1979; Gallin and Kirkpatrick, 1984 and Klesius et al., 1983). However, if it used

Table (4):
The effect of NDV-S-TF therapy on the protection rate of chickens challenged with virulent Newcastle Disease virus

Chicken group	Vaccination with NDV-vaccine	NDV-S-TF		No. of challenged chickens	Pattern of mortalities of chickens per days post challenge					No. of survivors	Protection rate
		treatment	Rout		day 4	day 5	day 6	day 7	day 8		
A	-	-	-	15	5	6	2	2	-	0	0%
B	+	-	-	15	-	1	-	-	1	13	86.6%
C	+	+	I/M*	15	-	-	-	-	-	15	100.0%
D	+	+	I/M**	15	-	-	-	-	-	15	100.0%
E	+	+	Oral@	15	-	-	-	-	-	15	100.0%
F	-	+	I/M	15	-	7	5	-	-	3	20.0%
G	-	+	Oral@	15	-	7	5	-	1	2	13.3%

@ NDV-S-TF treated chickens recieved - a dose of 5 vials/chick which represent dialysate obtained from a total of 5×10^8 lysed cells.

* NDV-S-TF was given simultaneous with the NDV-vaccine.

** NDV-S-TF was given 3 days before NDV vaccination.

as adjuvent, it potentiate the produced humoral immune responses, probably through the effect of transfer factor activated T-helper cells (Klesius and Kirkpatrick, 1983 and Huard et al., 1978). In the present work, similar results were obtained where adminstration of NDV-S-TF alone either orally or I/M did not stimulate significant antibody production against NDV-vaccine. However, the simultaneous adminstration of NDV-vaccine and NDV-S-TF, induced extraordinary immunopotentiation of the antibody production (See **Table, 3)** which directly took the form of a secondary immune response.

The specific and non-specific immunopotentiating effect of the NDV-S-TF was further documented by the challenge assay where the simualtaneous adminstration of Lasota NDV- vaccine and NDV-S-TF induced 100% protection, (irrespective of the route or the time of NDV-S-TF therapy), compared with 86.6% protection rate in those recieving the NDV-vaccine alone. Thus, the NDV-S-TF improved relatively the disease resistence in treated chickens.

However, in chickens treated with NDV-S-TF alone I/M or orally the protection rate was very low reaching to 20% and 13.6% respectively. Although this rate is low but it is still far better than the untreated chickens which showed a zero protection rate. Such low protection rate in the NDV-S-TF treated chickens can be attributed to many factors; firstly the proper immunizing dose of NDV-S-TF is not known and should be determined and secondly the TF preparation does not stimulate antibody formation which is known to play an important role in resistance to NDV infections.

In conclusion, the present work documented clearly the efficacy of TF preparation to transfer CMI, and declared its strong immunopotentiating effect on the humoral and cell-mediated immune response in

chickens against Newcasle disease virus. However, more investigations are required to determine the proper dose of TF preparation and the frequency of adminstration required to induce fully disease resistance.

SUMMARY

Efficacy of the adminstration of newcastle disease virus-specific-Transfer factor (NDV-S.TF) either orally or intramuscular injection in chickens was evaluated. The results revealed that NDV-S-TF potentiated the lymphocyte blastogensis response against PHA mitogen and NDV antigen; as well as induced extraordinary potentiation of the haemagglutinating antibody production against NDV in treated chickens than non-treated one.

Chickens innunized with NDV-S-TF and challenged were shoused good protection percentage then non-innunized birds.

REFERENCES

1. Amanullah, K.; Kirkpatrick, C. and Hill N. (1979): Third international symposium on transfer factor. Cell Immunol. 46 : 184-186.

2. Amanullah Khan, Beth Hansen, N.O. Hill, E. Loeb, A.S. Pardue and J.M. Hill. (1981): Transfer factor in the treatment of herpes simplex types 1 and 2. Dermatologica (Basel), 163 (2): 177-185.

3. Ascher, M.; A. Gottlieb and C. Kirkpatrick, (1978): "Transfer Factor": Basic properties, and clinical applications. Academic Press, New York.

4 . Ashorn, R.G.I.; K.M. Marnkela, A. Votila and K. J. E. Krohn, (1983): Augmentation of delayed - type hypersensitivity in antigen-primed guinea pigs by human dialyzable leukocyte extract. Acta Pathol. Microbiol. Immunol. Scand, Sect. C., Immunol., **90** (6): 331-338.

5 . Baram, P. and Condoulis, W. (1969): The in vitro transfer of delayed Hypersensitivity to Rhesus monkey and human lymphocytes with transfer factor obtained from Rhesus monkey peripheral white blood cells. J. Immunol., **104** (4): 769-779.

6 . Bouquet, J.F.; Devaux, B. and Moreau, Y. (1980): Immunization of breeders and layers against egg drop syndrome 76 (EDS-76) and Newcastle disease with an inactivated oily adjuvant vaccine. C.A.P. **165** (80): 1-10.

7 . Boyum, A. (1968): Isolation of mononuclear cells and granulocytes from human blood. Scand. J. Clin. Invest., **21**: 77-89.

8 . Brugh, M. Jr. (1978): A simple method for recording and analyzing serological data. Avian Dis., **22** (2): 362-365.

9 . Burger, D.R. and Wayburn, S. Jeter, (1971): Cell-free passive transfer of delayed hypersensitivity to chemicals in guinea pigs. Infec. & Immun., **4** (5): 575-580.

10. Catanzaro Antonio and Lynn Spitler, (1978): Results of Transfer factor therapy in cocci dioidomycosis. "Transfer Factor" Basic Properties and Clinical Applciations. (M. Ascher. A Gottieb and C. Kirkpatrick) Eds. Academic Press, New York.

11. Carey, John T., Michael M.; Lederman, Zahra Toossi; Kay Edmonds, Sally Hodder, Leonard H. Calabrese, Max, Ellner, (1987): Augmentation of skin test reactivity with lymphocyte blastogenesis in patients with AIDS treated with transfer factor. J.A.M.A., 575 (5): 651-659.

12. Chi, Davis S.; Michael, A. Palladino, Tom Romano and G Jeanette Thorbecke, (1982): Transfer of delayed hypersensitivity in the chicken. Dev. Comp. Immunol., 6 (3): 541-458.

13. Cruickshank, R.; Duguid, J.P.; Marmion, B.P. and Swain, R.H.A. (1985): Medical Microbiology, 12th Edition, Vol. II. The practice of Medical Microbiology Churchill Livingstone, Edinburgh, London and New York.

14. Fundenberg, H.H.; Wilson, G.B. and Smitth, C.L. (1980): Immuno-therapy with dialyzable leukocyte extracts and studies of their antigen-specific (transfer factor) activity. Proc. Virchow - Piruqet Med. Coc., 34: 3-87.

15. Fudenberg, H.H. (1985): "Transfer Factor" An update. Proceedings of the society for experimental Biology and Medicine, 178, 327-332.

16. Gallin, J.I. and Kirkpatrik C.H. (1974): Chemotactic activity in dialyzable transfer factor. Proc. Nat. Acad. Sci. U.S.A. 71, pp. 498-502.

17. Galnek, B.W. (1979): Hemagglutination inhibition antibodies against an adenovirus (virus 127) in white pekin ducks in the United States. Avian, Dis. 4 (22): 798-901.

18. Georgescu, Carmen. (1985): Effect of long-term therapy with transfer factor in rheumatoid arthritis. Rev. Rovm, Med. Interne, 23 (2): 135-140.

19. Giambrone, J.J.; Klesius, P.H. and M. Yu. (1983): Adoptive transfer of delayed wattle reactivity in chickens with a dialyzable leucocytic extract containing transfer factor. Poult. Sci. **62** (5): 767-771.

20. Huard. T.K.; Sabet, T. and Baram, P. (1978): In vivo effects of dialyzable leukocyte lysates (D.L.L.) Change in the response of murine spleen cells to T- and B- cells mitogens, spleen weight and morphology of splenic tissue. Clin. Immun., Immunopathol., **11**: 299-244.

21. Hudson, L. and Hay, F.C. (1980): Practical Immunology, 2nd edition. Blackwell Scientific Publications. Oxford, London.

22. Mostafa, H.A. (1991): Studies on Brucella-Specific M.V.Sc. Thesis, Fac. Vet. Med., Cairo Univ. Egypt.

23. Ishikawa, H. and Shirahatu, T. (1986): Application of glucose consumption assay for evaluating blastogenesis in bovine lymphocytes. Jap. J. Vet. Sci., **48** (1): 111-115.

24. Jailian, L.I. (1985): Human transfer factor. Sci. Siv. Ser. B. Chem. Biol. Agric. Med. Earth. Sci. 28 (4): 394-401.

25. Kita, Eigi, Yasushi Matsuda, Keiji Matsuda and Shuzo Kashiba, (1984): Separate transfer of mouse protection with delayed type Hypersensitivity with Salm. typhiurium transfer factor. Cell Immunol. **87** (2): 528-537.

26. Klesius, P.H. (1982): Immuno potentiation against internal parasites. Vet. Parasitol., 10, 239.

27. Klesius, P.H. and Charles, H. Kirkpatrick, (1983): Dialyzable leukocyte extract containing transfer factor-its future in Veterinary Medicine. Immunobiology of Transfer Factor. 129-140.

28. Klesius, P > H.; Hugh Fudenberg, and Charles, L. Smith, (1980): Comparative studies on dialyzable leukocyte extracts containing transfer factor. Microbiol. Infect. Dis.,3: 247-260.

29. Lawrence, H.S. (1955): The transfer in humans of delayed skin sensitivity to streptococcal M. and to Tuberculin with disrupted leucocytes. J. dClin. Invest., 34, 219-230.

30. Liburd, E.M.; Pabst, H.F. and Armstrong, W.D. (1972): Transfer factor in rat coccidiosis. Cell Immunol. 5, 487.

31. Lobuglio, F. Albert and James, A. Neidhart, (1976): Transfer factor A potential agent for cancer therapy. Med. Clin. of No. American, 60: 585-590.

32. Mayer, V.E. Gajdosova, M. Valaskova and C. Oravex. (1985): Antigen-specific transfer factor from mice immunized with an attenuated flavi virus augmentation of inducing activity in semi-purified splenocytic dialysate. Acta. Virol (Prague), 29 (1): 25-34.

33. Miksiewicz-Wasilewska, Helena, Anna Grubek - Jaworska, Michalina Plucinska, Barbara Podsiadlo, Pawel Krakowka and LUdwik Rzucidlo, (1981): Transfer factor in mucocutaneous candiddiasis treatment. Mater. Med. Pol., 13 (2): 108-112.

34. Nikonenko, B.V.; Marina, B.; Mezhlumova A. and A. M. Moroz, (1990): Local adoptive transfer of delayed type Hypersensitivity from Mycobacterium bovis (B.C.G.) infected mice. Folia Biol. (Prague)., **35** (4): 255-260.

35. Olson, G.B. and Drube, C.G. (1978): Influenza virus transfer factorin guinea pigs. J. Res. 24, 589.

36. Ramadan, A.; R. Soliman; Nehal, A. Afifi and I. Reda, (1991): Studies on factors influencing immune response in chickens. V. Effect of profiotieum "S" on immune resposne of chickens to Newcastle disease virus vaccine. J. Egypt. Vet. Med. Ass. **51** (1 & 2), 404-425.

37. Richard, J.; Graybill, Charles Ellenbogen, Douglas Drossman, Peterkaplan and Daniel Ethor. (1978): Transfer Factor therapy of Disseminated Histoplasmosis. "Transfer Factor" Basic properties and clinical applications (M. Ascher. A. Gottlieb and C. Kirkpatrick). Academic press. New York.

38. Rodae, E.; D. Viza, G.; Pizza, L.; Mastroroberto, J.; Phillips, C.; De Vinci and L. Barbara, (1985): Transfer factor for the treatment of Hepatitis B. surface antigen positive chronic active Hepatitis. Proc. Soc. Exp. Biol. Med., **178** (3): 468-475.

39. Ross, J.G. and W.G. Halliday, (1982): Investigations of transfer factor activity in resistance to Trichostrongylus colubriformis infectious in guinea pigs. J. Helmithol. **56** (1): 27-36.

40. Shimakura, Y.; Kuds, T.; Honjo, H.; Kitazawa, K. (1985): Glucose consumption test for peripheral transformation in "Shiba" goat. Res. Bull. Fac. Agric. Gifu Univ. Jap. **50**, 324-334.

41. Shusuke Tsuji, Shunsaku Oshima, Morio Oshiro and Takateru Izumi. (1964): Studies on the "Transfer Factor" of Tuberculin Hypersensitivity in animals. 39, 838-849.

42. Smith, Randall A.; Ahmed Esa and Mary Stiff. (1982): Transfer of Salmonella typhimurium resistance and delayed hypersensitivity with murine-derived transfer factor. Infect Immun., 36 (1): 271-276.

43. Wilson, G.B.; Poindexter, J.D. Fort and D.K. Ludden. (1988): De novo initiation of specific cell-mediated immune responsiveness in chickens by transfer factor (specific immunity inducer) obtained from bovine colostrum and milk. Acta Virol. (Prague) (Engl. Ed.).; 32 (1): 6-18.