ESTABLISMENT OF THE PREVALANCE OF PERSISTENTLY INFECTED CATTLE AND SHEEP IN ANATOLIA WITH FMDV

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SUMMARY

For the purpose of the determination of the prevalance of the animals persistentiy infected with FMDV, probang samples of 469 sheep and 147 cattle form 16 provinces in different districts of Anatolia have been collected. At the end of the isolation carried out with primary bovine thyroid (BTY) cell cultures and IB-RS 2 cell-line cultures and identification by CFT and/or ELISA the carrier rate in sheep population has been determined 16.8 % and in cattle population 18.4 % . The number of carrier animals in Middle-East and South-East Anatolia was more then the westren and northern parts. 4 sheep have been determined both A and O type FMDV carriers. It has been confirmed that BTY cells were more susceptible to FMDV field samples than IB-RS2 cell-line.

Nevertheless, by taking both the disadvantages of BTY cell culture system and the possible masking of FMDV particles by enteroviruses into consideration it has been recommended to search and adapt other susceptible system to the laboratory.

ÖZET
ANADOLU'DA ŞAP İLE PERSİSTE ENFEKTE SIĞIR VE KOYUNLARIN PREVALANSININ TESBİTİ

Şap virusu ile persiste enfekte hayvanların prevalansını tesbit etmek amacı ile, Anadolu'da 16 ilden 469 koyun ve 147 sığır probang numunesi toplantı. Primer dana tiroid (BTY) ve IB-RS 2 devamlı hücre kültürlerinde gerçekletirilen izolasyon ve CFT ve / veya ELISA ile gerçekleştirilen identifikasyon işlemleri sonucunda taşıyıcılık oranı koyunlar için %61,8 ve sığırlar için de %18,4 olarak belirlendi. Orta ve Güneydoğu Anadolu'daki taşıyıcı hayvan sayısı batı ve kuzey bölgelerine göre daha fazla idi. 4 koyunun hem A hem de O tipi şap virusu taşıyıcısı olduğu tesbit edildi. BTY hücrelerinin IB-RS 2 devamlı hücre kültürlerine oranla şap virusunun saha suşlarına daha duyarlı olduğu konusundaki önceki bulgular teyid edildi. Bununla beraber, BTY hücre kültürlerinin dezavantajları ve şap viruslarının hücre kültürlerinde enteroviruslar tarafından maskeleme olasılığı göz önüne alınarak yeni ve daha duyarlı test sistemlerinin laboratuvarlara adaptasyonu önerildi.

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INTRODUCTION :

Foot and mouth disease (FMD) is one of the most import animal disease. Early diagnosis of it is influential. The real danger is the case which the disease can not be diagnosed clinically. In those circumstance the endemic spreads invisible and in the same time the infected animals become virus carriers.

Inactivated FMD vaccines can protect the animals against clinical diseases however they can not prevent the virus from the replication in respiratory and upper alimentary tract (6, 19). In the case of the insufficient antibody level in blood as a result of active or passive immunity the variant strains appear which is an undesirable situation (6, 14, 26).

The carrier period varies according to the serotype of the virus and the animal species (14). This period is the longest in African buffalo it is folwowed by the cattle, sheep, goat and antelopes in turn of succession (2, 3, 13, 14, 27). That kind of carriage could not be detected in pigs (8).

In general the ruminants which produce and spread FMD virus (FMDV) regularly or time by time, four weeks later begin exposured to the infection are considered as carriers (14).

To realize the control and eradication of FMD, it is compulsory to determine the serotypes, subtypes and antigenic variants of the virus in the field. It is well known that the antigenic variant of a subtype can appear in the same area in the course of time. The variation of the virus particles in the oesophageal mucosa cells of the carriers has a role in this phenomenon (20).

The aim of this work is by determining the prevalance of the animals which are persistently infected with FMDV in Anatolia to contribute the designation of the control and eradication programmes of FMD.

MATERIAL AND METHOD :

Cell : Primary fetal calf thyroid (BTY) and pig kindey cell line (IB-RS2) cultures have been used for virus isolation.

Probang : Saliva colletion receptacles described by Suttmoller (Suttmoller, P, 1965) have been used.

Cell Cultures : Both BTY and IB-RS2 cell cultures were prepared in roller tube systems.

Sampling : Sampling of oesophageal - pharyngeal (0/P) fluid have been realized according to Kitching's technique (10).

Virus Isolation and Identification : 0 / P fluids been kept in liquuid nitrogen immediately after collection have been inoculated onto 3-5 days old BTY and 99-100 % confluent monolayer IB-RS2 cell-line cultures in the quantity of 0,5 ml/tube. Cytopathogenic effect (CPE) have been inspected for 3 days and CPE inspected cultures have been kept in the -20° C. Two more blind passages have been done for the culturs which no CPE had been observed. Virus identification have been realized with complement fixation test (CFT) and enzyme linked immunosorbant assay (ELISA).

Statistical Valuation : Individuals were selected by using the cluster sampling method for each group whose target population would be determined (1,

21). The sample sizes were taken to be 372 head for cattle and 117 head for sheep. The calculations were realized by taking the 2,65 % standard error into acount and with 95 % confidence limit. The percentage of the individuals in which the FMDV has been isolated, in target population were accepted as persistent rate.

By the acception of the estimated prevalance of the disease in sheep as 5 % and 1 % in cattle, the sample confidence interval

$$SE = \frac{CL}{d} \quad , \quad n = \frac{P\,\% \times Q\,\%}{SE}$$

where :

SE : standard error
CL : the table value of the confidence limit
n : sample size
p : number of positives
Q : number of netatives

Hence , the sample size was accepted to be 117 head for sheep and 372 head for cattle were separated into 3 age groups, the sheep into 2 age groups, such as;

for cattel (39 animals were aimed for each group) :
1 st group : 6 - 12 months of age,
2 nd group : 12 - 36 noths of age,
3 rd group : elder than 36 months of age.

for sheep (186 animals were aimed for each group) :
1 st group : 6 - 12 months of age.
2 nd group : elder than 12 months of ages.

However by taking the losses that might occur during the sampling and performing the tests into account, these amounts were excessed. The only exception for this situation was in the 1 st group of the sheep. Basicly, although at least 186 samples were taken because of being unable of finding animals in appropriate with the required conditions.

RESULTS :

469 sheep and 147 cattle have sampled from 16 provinces in Anatolia (Adana, Ankara, Antakya, Aydın, Bolu, Erzurum, İzmir, Karaman, Kars, Konya, Malatya, Manisa, Ordu, Samsun, Sivas, Zonguldak). Results of the probang tests have indicated that 15 sheep were A type, 60 sheep were O type and 4 sheep were both A and O type FMDV carriers; also 2 cattle were A type, 25 cattle were O type FMDV carriers (Table.1.). According to those figures 16.8 % of the sheep population and 18.4 % of the cattle population in Anatolia are persistently infected with FMD virus (Table. 1)

The dispersion of the carrier state according to the age groups was as fol-

lows : 15.6 % for the lambs younger than 1 year of age, 17 % for the sheep over 1 year of age, 20 % for the calves younger than 1 year of age, 13.3 % for 12-36 months of age cattle and 21 % for the cattle over 36 months of age (Table. 2.).

Persistent infection was more dense in East, Southern-east, and Central Anatolia : the rate was decreased through northern and western districts of Anatolia (Table 1).

In comparison of two tissue culture systems, 104 0/P samples were FMDV positive with BTY cells but only 90 of those were FMDV positive with IB-RS2 cell-line.

During three serial passages of each sample virus replication has been controlled first by CPE detection. In fact, totally in 125 sheep and 56 cattle semples CPE has been observed but CFT and ELISA results have indicated that in only 79 sheep and 26 cattle samples FMDV was present (Table 2).

DISCUSSION :

As most of other diseases early diagnosis improves the effectiveness of eradication campaigns. However in the case which clinical diagnosis is impossible, more risky situation appears. Infection spreads latently and most of the animals become virus carriers. Detection and estimation of virus carriers has a very important role in controlling the FMD (21).

There is only one study on the subject of the FMDV persistency in Turkey which covered only the cattle and sheep in Eastern Thrace (European part of Turkey) (22) while the aim of this study is to estimate the FMDV carrier rate and to check the general sitution of FMDV persistency in Anatolia (Asiatic Turkey).

In this survey the estimeted carrier rate for cattle was 18.4 % and for sheep 16.8 %. In a similar survey realized in Zimbabwe in 1985 the result was 18 % for African buffalos (3, 16). The persistent infection in the areas where the infection is enzootic (12) the rate varies between 20 - 52 % (22). Likevise in a field survey in Kenya the carrier rate has been estimated as 44 % for goats and 83 % for sheep in contrast in Egypt where previously no FMD outbreak had been observed the rate of persistently infected animals is decreasing from east, central and south-east of Anatolia through north and west. In fact it has a close relation with the prevalance of the disease. In suitable conditions even only one carrier animal is sufficient to infect its premises by aerosol route and to cause new outbreaks (5).

Carrier state has a correlation with animal species, virus strain and meteorological conditions (2, 3, 27). However there is no exact information about the distribution of FMDV persistence between several age groups. Also in this trial no relation has been estimated between the age of the animal an FMDV carrier rate.

It is well known that in the same district even in the same holding infection with two different serotypes of FMDV is possible. But up to now no mixed persistent infection of FMDV has been reported. In this study both A and O types of the virus have been isolated form 0/P samples of 4 sheep.

The preferable technique for FMDV isolation form 0/P fluid is the inoculation onto BTY cell cultures (4, 7, 9, 11, 18). However the troubles such as the difficulties to find foetus, the microbial contamination risk of primary cell cultures, the extreme manuplation for culture preparation and the loss of susceptibility to FMDV of frozen BTY cells (9) have directed us towards using IB-RS2, at least 4-5 days are needed for virus isolation form the 0/P sample with any kind of cell cultures. It makes that techique difficult to use for the purpose of the control of the animal movement. Recently polymerase chain reaction (PCR) tecnique seems to be the solution of this problem (12).

It is necessary to isolate and identify the virus particle to find out the persistently infected animal. That is, CPE alone is not the indicator of FMD virus, it must be confirmed by a serological method. Some other virus particles such as enteroviruses can also be present in 0/P fluid and they can be the cause of the CPE (22).

In this trial in 104 sample out of 174 CPE positive samples FMDV have been identified. On the other hand starting from the reality that FMDV in cultures can be masked by enteroviruses (22). It can be considered that some of the CPE positive but serologically negative samples probably also were FMDV infected. This hypothesis will increase the rate of persistently infected animals in Anatolia.

Table. 1 : The results of probang test realized with the 0/P samples received from several provinces.

	SHEEP		CATTLE		TOTAL SAMPLES	
PROVINCE	A TYPE	0 TYPE	A TYPE	0 TYPE	SHEEP	CATTLE
ADANA	4	14	0	6	34	18
ANKARA	0	0	0	0	9	13
AYDIN	1	0	0	0	17	7
BOLU	0	1	0	0	20	3
ERZURUM	6	2	0	0	51	0
HATAY	0	7	0	6	32	8
İZMİR	1	1	1	0	16	10
KARAMAN	0	22	0	3	45	5
KARS	6	1	1	0	51	5
KONYA	0	5	0	9	21	23
MALATYA	0	3	0	0	32	0
MANİSA	1	1	0	0	17	7
ORDU	0	0	0	0	20	4
SAMSUN	0	0	0	0	20	3
SİVAS	0	3	0	1	66	38
ZONGULDAK	0	0	0	0	18	3
TOTAL	19	60	2	25	469	147
PREVALANCE OF PERSISTENT INFECTION				%	16.8	18.4

Table. 2 : Distribution of the persistent infection according to the age groups

	SHEEP			CATTLE		
AGE GROUPS	POSITIVE SAMPLES	TOTAL SAMPLES	%	POSITIVE SAMPLES	%	TOTAL SAMPLES
1. GROUP (6-12 MTHS)	13	33	15.6	8	40	20.0
2. GROUP (> 12 MTHS)	66	386	17.0	-	-	-
(12-36 MTHS)	-	-	-	6	45	13.3
3. GROUP (> 36 MTHS)	-	-	-	13	62	21.0
TOTAL	79	469	16.8	27	147	18.4

Table. 3 : Quantity of positive samples which FMDV could be isolated and could not be isolated.

	FMD VIRUS - NEGATİVE SAMPLES	FMD VIRUS - POSITIVE SAMPLES	TOTAL
SHEEP	46	79	125
CATTLE	29	27	56

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