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OCCURRENCE OF PARAINFLUENZA TYPE –3 (PI-3) AND BOVINE HERPES VIRUS TYPE-I (BHV-I) VIRUSES (MIXED INFECTION) AMONG CAMELS

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Abstract

In a herd of 790 imported Djiboutian dromedary camels located at Suez quarantine station, respiratory signs accompanied by ocular discharges and weakness were observed.

PI-3 and BHV-I viruses were isolated on MDBK cell line from nasal and ocular swabs of diseased camels and from organs (lung, liver & spleen) of emergency slaughtered camels. Mixed infection with PI-3 and BHV-I were recorded.

PI-3 virus was identified by Haemagglutination Inhibition (HI) test with reference PI-3 antiserum and BHV-I by Indirect Fluorescent Antibody (IFA) technique using reference BHV-I antiserum.

Agar Gel Precipitation Test (AGPT) and Dot-Enzyme Linked Immune Sorbent Assay (ELISA) were applied on the above-mentioned samples for rapid detection of PI-3 and BHV-I viral antigens.

Also, 86 serum samples were collected from apparently healthy and diseased camels, tested by Virus Neutralization Test (VNT) for detection of neutralizing antibodies against PI-3 and BHV-I., and by HI-test for PI-3 heam agglutinating antibodies.

This is the first record for detection and isolation of PI-3 and BHV-I viruses (mixed infection) among camels in Egypt.

INTRODUCTION

Several viral agents are known to cause respiratory diseases in animals, of these, Parainfluenza type 3 (PI-3) virus and Infectious Bovine Rhinotracheitis (IBR) virus.

PI-3 virus is a member of the Family Paramyxoviridae, this family contains a large number of important human and animal pathogens which share the characters of the genus (Sakai *et al.* 1987).

IBR virus was classified among the members of Subfamily Alpha herpes virus and now commonly referred to as Bovine Herpes Virus type I (BHV-I) (Armstrong *et al.* 1991).

PI-3 virus acts as a predisposing cause for microbial invasion and multiplication in respiratory tissues and so resulting in pneumonia or shipping fever (Baz *et al.* 1982a). BHV-I often refers to IBR/IPV (infections bovine vulvovaginits). It is characterized by mucopurulent nasal discharge, conjunctivitis, general illness such as fever, depression and inappetece. The virus can also infect genital tract causing pustuler vulvovaginitis or balanopothitis (Baz *et al.* 1982b).

Concerning the first isolation of the above mentioned viruses in Egypt, PI-3 virus was first isolated in 1966 by Singh and Baz (1966) from buffalo-calves that were suffering from pneumoenteritis, and also isolated for the first time from cattle with respiratory illness at Suez quarantine station imported from Somalia (Singh and EL-Cicy, 1966). BHV-I was first isolated by Hafez and Frey (1973) from cases of pneumoenteritis in calves.

Trials were conducted by some investigators for isolation of PI-3 or BHV-I from camels, but without success (EL-Trabili 1979; Eisa, 1998 and Abou-Zaid, *et al.* 2001). Also Kouider *et al.* (1998) was not able to detect any antibodies in camel sera to BHV-I and they concluded that dromedary camels were not susceptible to BHV-I.

On the other hand, specific precipitating, neutralizing and haemagglutinating antibodies were detected to these viral agents by other researchers. EL-Trabili (1979) detected neutralizing and haemagglutinating antibodies against PI-3 virus in camel sera. Neutralizing antibodies against PI-3 and BHV-I were detected among two groups of camels without virus isolation (Eisa 1998). Also, HI antibodies against PI-3 virus were detected in camel sera, but without clinical signs (Afzal and Sakkir, 1994). Later, Precipitating and neutralizing antibodies against PI-3 and BHV-I in camel serum samples without history of vaccination or clinical signs were recorded (Abou-Zaid *et al.* 2001), and this was an indication of subclinical infection.

Our study explained the trial applied to explore occurrence of PI-3 and BHV-I (mixed infection) among imported Djiboutian dromedary camels.

MATERIALS AND METHODS

A. Materials

(1) Samples

Blood samples were obtained from 52 apparently healthy and 34 diseased camels imported from Djibouti and Rept at Suez quarantine station. The serum samples were collected after centrifugation of the clotted blood and stored at – 20°C until tested for PI-3 & BHV-I viral antibodies.

Nine nasal and 5 ocular swabs were collected from diseased camels, as well as portions from lung, liver and spleen were obtained from 4 emergency slaughtered camels. Swabs and tissues were taken, prepared aseptically and kept frozen at – 70°C until tested for virus isolation and detection of PI-3 & BHV-I antigens.

(2) Cell culture

Madin Darby Bovine Kidney (MDBK) cell line, a permanent cell line of MDBK cells was supplied by the National Veterinary Disease Laboratory (NVDL) in USA. This cell line was propagated and maintained in Animal Health Research Institute, Dokki, Giza. Cells were grown in MEM supplemented with 10% bovine serum, and used for virus isolation and preparation of BHV-I and PI-3 viral antigens.

(3) Viruses

IBR virus: Abu Hamad strain isolated by Hafez (1973).

PI-3 virus: Strain 45, isolated and identified by Singh and Baz (1966).

These two viruses were used in NT.

(4) Viral antigens

Positive and negative viral antigens were locally prepared from infected and non-infected MDBK cell culture with reference viruses according to (Robert *et al.* 1979). The viral antigens were used in AGPT, HI-test and Dot –ELISA technique.

(5) Reference positive immune sera

Standard reference positive hyper immune sera of BHV-I and PI-3 were supplied by Serum and Vaccines Production and Research institute, Abbasia, Cairo, Egypt. These

reference sera were used in AGPT, HI and Dot- ELISA.

(6) Enzyme Conjugate

Antibovine horse reddish peroxidase conjugate was supplied by Sigma immuno-chemicals used in Dot-ELISA.

Antibovine fluorescin isothiocyanate conjugate was supplied by Sigma immuno-chemicals used in IFA technique.

B. Methods

(1) Isolation

The methods of infecting the cell culture of MDBK with prepared nasal and ocular swabs and organs extracts were adopted according to Pierre and Michel (1993). The inoculated tissue cultures were observed daily for the development of cytopathic effect (CPE).

(2) Identification of the causative agent

a) Haemagglutination inhibition (HI) test

The test was carried out according to Lennette and Schmidt (1964) for identification of the isolated PI-3 virus from nasal and ocular swabs as well as organ extracts.

b) Indirect Fluorescent Antibody (IFA) technique

The test was done according to Mayewska *et al.* (1984) for identification of BHV-I on the infected MDBK cells.

(3) Detection of viral antigens

a) AGPT: The prepared nasal & ocular swabs and organ extracts were tested against reference antisera of PI-3 and BHV-I by AGPT according to the method described by Ouchterlony's (1968).

b) Dot- ELISA was applied according to Hawkes *et al.* (1982) for detection of BHV-I & PI-3 viral antigens in the prepared swabs and organ extracts.

(4) Detection of viral antibodies

a) Virus Neutralization Test (VNT) was conducted for detection of specific

PI-3 and BHV-I neutralizing antibodies in camel serum samples according to (Piere and Michel, 1993).

b) HI – test was carried out according to Lennette and Schmidt (1964) for detection of specific PI-3 haemagglutinating antibodies in the collected serum samples.

RESULTS AND DISCUSSION

Clinical investigations

The clinical examination of 790 imported Djiboutian dromedary camels aged between 4-5 years revealed that 34 of them were suffering from respiratory distress characterized by cough, dyspnea, serous to mucopurulent discharges accompanied by lacrimation and general depression.

P.M. examination

Four camels were emergency slaughtered. P.M. examination of these cases showed general emaciated carcasses with congestion in the internal organs mainly lung, liver, spleen and intestine.

Laboratory diagnosis

Isolation & Identification of the causative agent

Isolation from swabs and organs tissues was conducted on MDBK cell culture, the inoculated cells were observed daily for the presence of CPE, the cytopathic inducing viral agents were identified by IFA technique using reference BHV-I antiserum for BHV-I, and by HI-test using reference PI-3 antiserum for PI-3 virus. The results were illustrated in Table I. Sixteen cytopathic inducing viral agents were isolated out of 26 different samples (swabs and organ tissues) (61.5%). Some of these isolates induced typical CPE characteristic for BHV-I in the form of aggregation of MDBK cells giving the grape-like appearance 72-h post-inoculation. Such changes gave suspicion for the presence of BHV-I among these isolates. (Hafez, 1973, Hafez and Frey, 1973 and Baz *et al.,* 1982b). Other isolates induced CPE in the form of cell rounding and anastomosis of the cells and sheet detachment, such changes gave suspicion for the presence of other viruses.

The sixteen isolates included were 6 from nasal swabs, 3 from ocular swabs of diseased camels and 4 from lung, 2 from liver and one isolate from spleen tissues of 4 emergency slaughtered camels.

Out of 16 cytopathic viral agents 13 BHV-I isolates (50%) could be identified by IFA technique using reference BHV-I hyperimmune serum and 5 PI-3 isolates (19.2%) were identified by HI-test using reference PI-3 hyper immune serum.

Mixed infections between BHV-I and PI-3 viruses were detected in 4 samples (2 nasal, 1 ocular swab and one from lung tissues) out of 16 cytopathic viral agents (15.4%).

The results of detection of BHV-I & PI-3 antigens in diseased & emergency slaughtered camel samples (swabs & organs extracts) by AGPT & Dot - ELISA are summarized in Table (2) as follows:

By AGPT: Out of 26 samples, 19 gave positive reaction (precipitin line) (73.1%) for BHV-I, and 7 samples were positive for PI-3 (26.9%).

By Dot. ELISA: Out of 26 samples, 22 of them were positive (84.6%) for BHV-I and 10 samples were positive (38.5%) for PI-3.

Mixed infections were also detected in the collected swabs or organ extracts either by AGPT or Dot-ELISA. By AGPT: There were 3 samples (2 nasal swab and one lung extract) that gave precipitin line against BHV-I reference antiserum and PI-3 reference antiserum (11.5%). By Dot-ELISA: Out of 26 samples, 9 of them (4 nasal swabs, 2 ocular swabs, 2 lung extracts and one liver extract) gave positive reaction for BHV-I and PI-3 (mixed infection) (34.6%).

Regarding the BHV-I & PI-3 antibodies in apparently health and diseased camels serum samples, VNT was carried out for detection of specific neutralizing antibodies for these viral agents (Eisa, 1998) and HI-test was used for PI-3 haemagglutinating antibodies for its specificity (El-Trabili, 1979 and Abou-Zaid *et al.,* 2001). The results of these are shown in Table (3) where 86 from camel serum samples, 39 (45.3%) and 32 (37.2%) were positive by VNT for BHV-I and PI-3, respectively. By the test of choice for PI-3 (HI test): 36 serum samples were positive for PI-3. In Table (3), mixed occurrence of BHV-I and PI-3 viral antibodies was demonstrated by VNT either in apparently healthy (3 out of 52 serum samples), or in diseased camels (7 out of 34 serum samples) with a total of 10 samples (11.6%). Simillar results were reported by El-Trabili (1979), Moussa *et al.* (1990), Afzal and Sakkir (1994) and Abou-Zaid (2001).

Although, there is no sufficient data concerning these diseases in camels, our obtained data in general indicate clearly that camels can suffer from the infection of BHV-I and/or PI-3 viruses which may produce inapparent or clinical respiratory form among

them. So, in conclusion, camels can most probably play a significant role in the epizootiology of these viral diseases.

Table 1. Results of isolation and identification of BHV-I & PI-3 viruses from diseased and 4 emergency slaughtered camels.

Animal Species	Condition	Samples tested		No. of samples with CPE	Virus identification		
		Type	No.		IFA BHV-I	HI PI-3	Mixed BHV-I + PI-3
Dromedary Camels	Diseased	Nasal swabs	9	6	5	2	2
		Ocular swabs	5	3	3	1	1
	4 emergency slaughtered camels	Lung	4	4	3	1	1
		Liver	4	2	1	1	-
		Spleen	4	1	1	-	-
Total	-	-	26	16	13	5	4
Over all percent	-	-	-	61.5%	50%	19.2%	15.4%

Table 2. Detection of BHV-I and/or PI-3 antigens in diseased and 4 emergency slaughtered camels.

Animal species	Condition	Samples		AGPT			Dot-ELISA		
				Positive			Positive		
		Type	No	BHV-I	PI-3	Mixed BHV-I + PI-3	BHV-1	PI-3	Mixed BHV-1 + PI-3
Dromedary camels	Diseased	Nasal swabs	9	9	3	2	9	4	4
		Ocular swabs	5	4	1	-	5	2	2
	4 emergency Slaughtered Camels	Lung extract	4	4	2	1	4	2	2
		Liver extract	4	2	1	-	3	2	1
		Spleen extract	4	-	-	-	1	-	-
Total	-	-	26	19	7	3	22	10	9
Overall percent	-	-	-	73.10%	26.90%	11.50%	84.60%	38.50%	34.60%

Table 3. BHV-1 and PI-3 specific antibodies in apparently healthy & diseased camels serum samples

Animal species	Condition	Tested sera	VNT			HI
			+ve BHV-I	+ve PI-3	Mixed BHV-I & PI-3	+ve PI - 3
Dromedary Camels	Apparently healthy	52	17	14	3	16
	Diseased	34	22	18	7	20
Total		86	39	32	10	36
Over all percent		-	45.30%	37.20%	11.60%	41.90%

REFERENCES

1. Abou-Zaid, A., K.A. Iman Kassem., M. Hussein and S.M. Zeidan. 2001. Bovine virus diarrhoea, Parainfluenza-3 and Infectious bovine rhinotracheitis antibodies in native and imported camel sera. J. Egypt. Vet. Med. Ass., 61 (6c):15-23.

2. Afzal. M. and M. Sakkir. 1994. Survey of antibodies against various infectious disease agents in racing camels in Abu-Dhabi, United Arab Emirates. Revue. Scientifique. et –Technique.O.I.E. 1994, 13:3. 787-792.

3. Armstrong, J.A., H.G. Pereira. and C.R. Anderws. 1991. Observations on the virus of IBR and it's affinity with the herpes virus group. Virology, 14 : 276-280.

4. Baz, I. T., M. M. Taha, H.Monira. Zahran. 1982a. Respiatory viral newborn calf diseases in Egypt. I-Studies on the role of PI-3 infection and colostral antibodies. Agri, Res. Review, 60 (7): 123-139.

5. Baz, I. T, M. M. Taha, Monira, H. Zahran and El-Dobeigy Aida, I 1982b. Respiratory viral newborn calf diseases in Egypt. II-Studies on the role of IBR infection and colostral antibodies. Agri. Res. Review, 60 (7): 105-122.

6. Eisa, M.I.1998. Serological survey against some viral diseases in camel in Sharkia governorate - Egypt. Proceeding of the 3rd Annual Meeting for Animal production under Arid condition, 1998, United Arab Emirats university. 167-173.

7. El-Trabil, M.M .1979. Studies on Parainfluenza-3 virus in camels. Thesis (M.Sc.) Fac. of vet. Med. Cairo university.

8. Hafez, S. M. 1973. Isolation and identification of infectious bovine rhinotracheitis (IBR) virus. Proc. 10th arab. Vet. Cong. Cairo.

9. Hafez, S. M. and H. Y.Frey. 1973 . Serological evidence for occurence of BVD (MD) and IBR in Egypt. Bull. Epiz. Dis. Afric. 21 (1) 5-10.

10. Hawkes, R., N. Evelyn. and J. Gordon. 1982. A dot immunobinding assay for monoclonal and other antibodies. Analy. Biochem., 119 : 142-147.

11. Kouider, A. s F. Schenjel, and W.Wernery. 1998.The map of camel diseases in Syria, Camel Newsletter (15).

12. Lennette, E. H. and N. J. Schmidt, 1964. Diagnostic procedures for viral and rechettsial diseases. 3rd Edition.Am. Public. Hlth Assoc. Inc., 1790. Broadway, New York, N. Y. 10019.

13. Mayewska, H. J. Zmudzinskik and Z. Baczynski,. 1984. The use of different immunofluorescence methods for ideutification of IBR/IPV and BVD-MD viruses isolated from pathological material. Bull. Vet. Ins. Pulawy, 27: 1-4.

14. Moussa, A. A., M.S. Saber, E., Nafie, M.A., Shalaby, N. N., Ayoub,S., El-Nakashly A.Y., Mohsen, H. M., Madbouly, A. A., El-Sanousi, M. M.,Fatehia, A.,Sami, I. Allam, and I. M Reda, 1990. Serological survey on the prevalence of BHV-I in domestic animals in Egypt. Vet. Med. J. Giza, 38 (1): 87-94.

15. Ouchterlony, O. 1968 Handbook of immunodiffusion and immunoelectrophoresis. Ann. Arbor. Mi: Ann. Arbor Science Publishers, Inc.

16. Piere, P. and, T. Michel. 1993. Methods and Techniques in Virology. PP.19-26.

17. Robert, H. P., W. K. Hyun, D. B. Carl, D. B., Mare R. Linda, L. G. John and M.C Robert. 1979. Diagnostic procedure for viral, richettsial and chlamydial infection. Washingto, DC American Public Health.

18. Sakai, Y., S. Suzu, T. Shioda. and H. Shibuta. 1987. Nucleotide sequence of bovine PI-3 virus genome : it's 3 end and the genus of NPPC and M proteins. Nucleic Acids Res., 15: 2929.

19. Singh, K. V. and I. Baz, Thanaa. 1966. Isolation of Parainfluenza-3 virus from water buffalo in Egypt. Nature, 210 : 656-657.

تواجد فيروسى البارا – إنفلونزا النوع ٣ وفيروس الهيريس النوع ١ فى الجمال

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تم رصد أعراض تنفسية مصحوبة بإفرازات عينية وضعف عام فى قطيع من الجمال عدده ٧٩. جملا بالمجر البيطرى بالسويس تم إستيرادها من جيبوتى .

وعلى خلايا MIDBK تم عزل فيروسى البار أنفلونزا النوع ٣ وفيروس الهربس النوع ١ من المسحات الأنفية والعينية المأخوذة من الحيوانات المريضة وكذلك من الرئة ، والكبد والطحال للجمال المذبوحة إضطرارياً . وبإستخدام إختبار مانع التلزن تم التعرف علي فيروس البارا أنفلوزا النوع ٣ وبإختبار الفلورسنت الغير مباشر تم التعرف على فيروس الهربس النوع ١ كذلك تم إختبار عينات المسحات وخلاصة الأعضاء للكشف عن انتيجين هذين الفيروسين بواسطة اختبار الترسيب بالأجار وإختبار الاليزا النقطية وقد كانت هناك عدوى فردية ومختلطة بهذين الفيروسين .

ايضاً تم تجميع عدد ٨٦ عينة سيرم من الجمال المخالطة إما سليمة ظاهرياً أو مريضة واختبرت هذه العينات بإختبار التعادل الفيروسى للكشف على الأجسام المناعية المعادلة لهذين الفيروسين وكذلك بإختبار مانع التلزن للكشف على الأجسام مانعة التلزان لفيروس البارا أنفلونزا وسجلت النتائج تواجد أجسام مناعية للفيروسين .

وهذا هو أول تسجيل لعزل فيروس البارا أنفلونزا النوع ٣ وفيروس الهربس النوع ١ (عدوى مختلطة) بين الجمال فى مصر .