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RAPID DIAGNOSIS OF BOVINE ADENOVIRUS SUBGROUP 1 INFECTIONS IN CATTLE WITH ACUTE RESPIRATORY DISEASE BY DIRECT IMMUNOFLUORESCENCE TECHNIQUE

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Akut Solunum Sistemi Enfeksiyonu Bulguları Gösteren Sığırlarda Bovine Adenovirus Subgrup 1 Enfeksiyonlarının Direkt İmmunofloresan Testi ile Çabuk Teşhisi

Özet: *Bu çalışmada, 8 farklı sürüde bulunan solunum sistemi enfeksiyonu bulgularına sahip 64 sığıra ait nasal swap materyalinden hazırlanan epitel hücre preparatları direkt immunfloresan tekniği ile Bovine Adenovirus subgrup 1 antijenleri yönünden kontrol edildi. Örneklenen 8 sürüden 2 adedinde ve 64 sığırdan 3 adedinde Bovine adenovirus enfeksiyonu saptandı (BAV tesbiti bir sürüde 2/5, diğerinde 1/6).*

Elde edilen bulgular Bovine adenovirusların sığırların solunum sistemi enfeksiyonlarında önemli bir etiyolojik ajan olabildiğini ve nasal swap örneklerinde antijen saptanması esasına dayanan direkt immunofloresan tekniğinin BAV'ların neden olduğu solunum sistemi enfeksiyonlarında etiyolojik ajanın saptanmasında kısa sürede uygulanan,duyarlı bir yöntem olduğunu ortaya koydu.

Anahtar Kelimeler: *Adenovirus enfeksiyonu, direkt immunofloresan, sığır*

Summary: *Nasal cells extracted from nasal swabs obtained from sixty four cattle with signs of respiratory disease ,from 8 different herds, were tested for Bovine adenoviruses subgroup 1 antigens using direct immunofluorescence technique.*

BAdV antigen positive samples were detected in two of eight herds examined. Of the 64 individual diseased cattle, three were found positive for BAdV subgroup 1 viral antigen (2/5 samples from one herd, 1/6 from the other).

The findings reveal that BAdVs may be an important causative agent in cattle respiratory disease and direct immunofluorescence technique as a rapid method, based on the detection of antigen in nasal swab samples, has been used to establish the viral aetiology of acute respiratory disease caused BAdVs subgroup 1 .

Key words: *Adenovirus infections,direct immunofluorescence technique,cattle*

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Introduction

Bovine adenoviruses (BAdV) are members of the genus *Mastadenovirus* of the family *Adenoviridae* . Two distinct subgroups of these viruses have been identified in cattle. Subgroup 1 BAdV is represented by serotypes 1, 2, 3 and 9, and subgroup 2 by serotypes 4 to 8 (4). An additional isolate from New Zealand has been designated bovine adenovirus-10 (1). Subgroup 1 BAdV possess a subgroup-specific antigen that is shared with other mammalian adenoviruses. These viruses replicate extensively in epithelial cells of the mucous membranes of conjunctiva, nasal cavity, throat, bronchi or intestinal tract.

Bovine respiratory disease involves complex interactions between infectious agents and various physiological and environmental factors. Among the viruses, bovine respiratory syncytial virus, parainfluenza type-3 virus, infectious bovine rhinotracheitis virus, and bovine adenoviruses have all been ascribed an important primary role in the pathogenesis of respiratory tract disease in cattle (3, 6, 14). The identification of the aetiological agent in pneumonia cases is often difficult. Traditional virus isolation technique in cell culture has proved useful (5,13) but time, maintenance costs, and the need for confirmatory tests for further identification of isolated viruses represent considerable disadvantages. Some viruses are difficult or impossible to grow in conventional cell culture systems and can only be detected by immunological methods. Immunofluorescence (IF) examination of diseased tissue, cells aspirated from bronchial lavages,or cells extracted from nasal swabs is the most efficient method of detecting many respiratory viruses in sick animals (7, 11, 12, 15). The method has also been extensively used in the human medical field (9, 10, 13).

The purpose of this study was to investigate the role of BAdV as aetiological agents in cattle with respiratory disease and to determine the efficacy of the direct IF technique for detection of BAdV in nasal epithelial cell smears.

Material and Method

Sampling animals: Sixty four cattle with signs of respiratory disease, from 8 different herds, were examined. Respiratory disease was defined as the presence as at least one of the following signs: nasal discharge, abnormal breathing, respiratory distress, increased respiratory rate, cough.

Nasal swap samples: Nasal swab samples were collected in PBS during the acute phase of disease. They were vigorously vortexed before being centrifuged at 1000 rpm at +4°C as soon as possible after collection. The cell pellets were used for immunofluorescence technique.

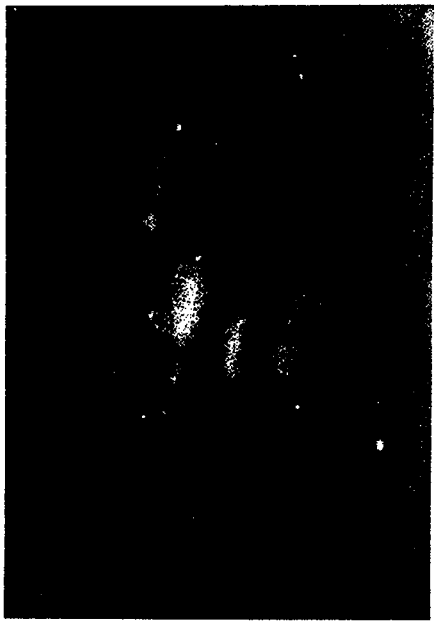
Immunofluorescence technique: The cell pellets were resuspended in a small volume of phosphate buffered saline (PBS) and washed twice with PBS. One drop of the suspension was dried on a microscope slide at room temperature for 30 min. After the smears were fixed with acetone for 10 min, the smears were washed three times in phosphate buffered saline over 15 minutes,then air dried and overlaid with a predetermined working dilution (1/20 in PBS, PH. 7.2) of BAdV-specific fluorescent antibody conjugate (Veterinary Laboratories Agency, Weybridge, UK). This was incubated in a humidified chamber for 30 min. at 37°C. After washing three times in PBS and once in distilled water the smears were air dried and mounted in buffered glycerol. Smears showing one or more cells stained with characteristic nucleic and cytoplasmic fluorescence were considered positive. As a control cell cultures infected with BAdV type-1 were used.

Results

BAdV antigen positive samples were detected in two of the eight herds examined. Of the 64 individual diseased cattle, three were found positive for BAdV subgroup 1 viral

antigens (2/5 samples from one herd, 1/6 from the other). The immunofluorescent staining in the nasal swab smears was for the most part diffuse cytoplasmic. The cells were oval or round in shape, mainly epithelial (Figure 1).

Figure 1.Large clump of fluorescing nasal epithelial cells X160



Discussion

Serological data suggest that bovine adenoviruses are widespread all over the world and may be causally related to some outbreaks of respiratory disease in cattle. BAdV3 was isolated in Turkey from cattle with respiratory tract disease (5). Alkan et al. (2) reported that serological prevalance rates for BAdV-1, BAdV- 2 and BAdV-3 type infections in cattle in Turkey were 23.7%, 35.2 % and 12.0 % respectively, indicating that exposure to these viruses is common.

Although direct IF for adenovirus antigen in clinical specimens has been widely used in human medicine (9,13) it is less frequently reported from veterinary diagnostic laboratories, even though the method has proved very successful for other bovine respiratory pathogens (11,15) Edwards et al. (7) reported that BAdV antigen positives had been found by direct IF in 10.1% of 79 respiratory disease outbreaks investigated by Veterinary Investigation Centres in England and Wales, while Haralambiev et al. (8) identified adenovirus antigen by IF in lung lavages from 3/42 pneumonic calves.

Few veterinary laboratories examine routinely for adenoviruses in bovine respiratory disease. The results from this study suggest it may be worthwhile adding adenoviruses to the range of antigens tested in diagnostic laboratories. By adopting such an approach the role of adenoviruses in the bovine respiratory disease complex may become clearer.

The other result from this study reveal that direct immunofluorescence technique as a rapid method, based on the detection of antigen in nasal swab samples, has been used to establish the viral aetiology of acute respiratory disease caused BAdVs subgroup 1. The limitation of IF and other immunoassay methods for diagnosis by antigen detection is that they only the detect the organisms which are specifically sought.

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