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Short Communication

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The Detection of an Unidentified Type of Adenovirus in the Stools of Calves with Weak Calf Syndrome by Use of a Commercial Kit Designed for the Detection of Human Adenoviruses

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With 1 table

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Summary

An outbreak of polyarthritis in newborn calves in a large collective dairy herd was characterized by intra-articular blood-tinged synoviae, blood tainted faeces and massive subcorneal haemorrhages. Faecal samples from eight clinical newborn cases, 10 from unrelated dairy farms and 10 faecal samples from healthy calves were examined by the Rida® Quick rotavirus/adenovirus-combi test . A specific adenovirus antigen precipitin-line was seen in the reaction in all the faecal samples from the diseased calves ($n = 8$), while all the others ($n = 20$) were negative. In addition, the same positive reaction was noted when one aqueous humor and two synovial samples were tested with this kit. Several other enteropathogens were found sporadically, but no conclusive significance could be attributed to their presence. Bovine viral diarrhoea and infectious bovine rhinothracheitis viruses as well as *Chlamydia* spp. and *Mycoplasma* spp. were not involved in this episode.

Introduction

Diseases of young calves in Israel are most often characterized by enteritis [neonatal calf diarrhoea (NCD)], pneumonia or pneumo-enteritis (Brenner et al., 1993, 2000;). There have been outbreaks however, where polyarthritis (PA) was the major presenting sign in Israel (Meirom et al., 1996) and elsewhere (Scott, 1995). The PA complex in newborn calves may involve various aetiological agents depending on environmental and management factors (Cutlip and McClurkin, 1975; McClurkin and Coria, 1975; Scott, 1995; Meirom et al., 1996).

A particular PA syndrome was reported by (Cutlip and McClurkin, (1975), McClurkin and Coria, (1975) and Stauber et al. (1975)), which was suggested to be one of the multiple features of the weak calf syndrome (WCS) (Brenner et al., 1998). This clinical phenomenon was labelled WCS as the affected calves were usually weak at birth, reluctant to suckle colostrum or milk, were unable to rise without assistance and when forced to move, walked stiffly, suggestive of painful joints.

Adenovirus was isolated from affected animals and the syndrome was reproduced experimentally using a viral isolate that was recovered during one of the outbreaks (Cutlip and McClurkin, 1975).

Adenoviruses have been associated with or related to bovine pneumonia, enteritis or pneumo-enteritis especially in young or newborn calves (Kahrs, 2001).

In this communication, we report an outbreak of PA in newborn calves from a large collective farm that resembled the WCS published 30 years ago (Cutlip and McClurkin, 1975) and McClurkin and Coria, 1975). Similar clinical cases were first noted in Israel almost a decade ago, although no conclusive results were obtained about the causative agent (Brenner et al., 1998).

Bovine adenoviruses were initially identified when a virus that was antigenically related to a human adenovirus was isolated (Klein et al., 1959, 1960).

Another group of investigators has reproduced clinical disease in susceptible young calves by infecting them with adenovirus isolated from sheep (Belák et al., 1977; Tùry et al., 1978).

In this current outbreak, however, bovine adenovirus involvement was ascertained by using an indirect laboratory technique that is generally used to confirm the presence of adenovirus in the faeces of diarrhoeic children.

Materials and Methods

In cases of neonatal diarrhoea complex, faecal samples are routinely assayed at the Kimron Veterinary Institute (KVI) for the following causative agents: *Enterotoxigenic Escherichia coli (K99+/F5) (ETEC)*, rotavirus and coronavirus, *Cryptosporidium parvum* and *Coccidia* and *Salmonella* spp. The *Salmonella* assay is routinely performed up to the identification of the *Salmonella* group and is further typed serologically in the Central Laboratory for Enterobacterriacae, Ministry of Heath (Brenner et al., 1993, 2000;). In addition, the faecal culture includes gastrointestinal fungi, such as *Candida* spp. (Elad et al., 1998, 2002;). Upon specific request, intestinal *Chlamydia* spp. are investigated by isolation, immunofluorescence or PCR (Brenner et al., 2001) as is the case when PA is suspected, where *Chlamydia*, *Mycoplasma* and other bacteria that might be involved in neonatal PA are also taken into consideration (Meirom et al., 1996).

The newborn rearing regimen

Most farms have adopted the colostral regimen that is generally recommended in Israel (Brenner, 1991), which

consists of feeding the newborn calf about 2 l of first pooled colostrum up to 4 h after calving. This pool is formed by collecting the first colostrum from at least five adult milking cows with more than 80 mg/ml of total IgG as measured with colostrometers by the attendants. The ration is completed with an additional meal, totalling at least 4 l of the pooled colostrum during the first 24 h of life.

On this farm, however, the dams are generally left with their offsprings in individual delivery pens with dry straw bedding, which is renewed for each new dam that is introduced. The newborn and its mother remain together for approximately 8 h, after which the calf is transferred to a neonatal indoor parlour where it is housed in an individual pen raised 40 cm above a concrete floor. When the calf is 10 days old, it is transferred to an individual outdoor pen where the ground is covered with absorbent volcanic ash.

Water is introduced in a bucket *ab libitum* from day two, while the neonate continues to receive colostrum till day 3. From day 4, it receives a milk substitute. Dry food is offered from day 4, which includes mainly, a mixture of milk substitute (powder) and dry cottonseeds, although the milk substitute remains the principal source of energy until weaning.

Case report

On May 1, 2003, four faecal samples from diarrhoeic calves between 7 and 10 days old, from a large collective dairy farm, arrived at the KVI for routine diagnosis of ruminant associated enteropathogens.

The local veterinary practitioner stated that from the first meal, immediately after birth, the newborn calves were reluctant to suckle or drink colostrum and therefore, the breeder resorted to feeding them by means of a gastric tube for several days. Improvement was seen after intravenous treatment with electrolytes and glucose but approximately 12% mortally was recorded. Five of 40 female calves died during this outbreak while the male calves were culled immediately after birth. Diarrhoea was noted from the third to fourth day after birth. Antibiotics were administered to the diarrhoeic calves at the beginning of the outbreak and were discontinued because the veterinarian noted no improvement.

The bacteriological assays were carried out on the faecal samples immediately upon arrival at the KVI and the samples were refrigerated overnight for rotavirus and corona virus tests on the following day. Twenty-four hours later, while awaiting further diagnostic procedures, a white-brown patina was noted on the surface of the faecal samples. This unusual phenomenon was of particular interest and a visit was made to the farm's neonatal unit to investigate possible sources of contamination.

A single carcass was brought for necropsy, but because of autolysis it was not suitable for histological examination. Aqueous humour was drawn for antigenic assay.

Clinical findings on the farm

The clinical manifestations previously describe by the local veterinarian were corroborated upon the visit. The affected calves were unusually weak at birth, reluctant to suckle colostrum and milk, were unable to rise without assistance and when forced to move, walked stiffly, suggestive of painful joints. Two newly born calves and their dams were still present in the individual calving pens situated within the calving premises.

These two calves were extremely weak. Stifle, hock, carpal and tarsal joints were enlarged and on palpation of the adjacent tissues, sub cutaneous oedema and crepitations were felt. From each enlarged joint, about 10 ml of blood-tinged synovial fluid was easily aspirated, in which fibrin clots were seen shortly after withdrawal. Ecchymotic haemorrhages were noted on the sclera.

Marked joint swelling was observed in other calves that were kept in individual pens, and in several cases a fluid filled swelling distal to the carpus denoted seepage of the synovia from the joint cavity. The fresh faeces on the ground were dotted with drops of frank blood. Older calves between 2 and 8 weeks showed alopecia around the joints, suggestive of a previous episode of polyarthritis.

Microbiological examinations

Tests for detecting intestinal yeast, enteric bacteria (F5+/K99 ETEC, *Salmonella* spp.) viruses (rotavirus, coronavirus) and protozoa (*Cryptosporidium parvum*) were performed on all faecal samples, as previously described (Brenner et al., 1993, 2000). Attempts to isolate and to identify *Mycoplasma* spp. were carried out as described by Levisohn et al., (2004). Acetone fixed synovial and faecal smears were examined by direct immunofluorescence using monoclonal fluorcein-isothionate conjugated antibodies to a group specific chlamydial antigen (Cellabs, Brookvale, NSW, Australia).

Adenovirus examination

A commercial kit Rida® Quick rotavirus/adenovirus-combi test (R-Biopharm, AG, Darmstadt, Gemany) was used for the detection of adenovirus in faeces, in synovial fluid and in aqueous humor. The procedure was carried out as recommended by the manufacturers.

The information provided by r-biopharm states that the relevant reagent incorporated in the one step immuno chromatographic assay for identification of adenovirus is designated as 2Hx-2−, a monoclonal immunoglobulin G2a (IgG2a), directed against adenovirus group-specific antigen.The manufacturer claims that the sensitivity and specificity are 100% and 99%, respectively.

In order to corroborate the ability of Rida® Quick rota/adeno-combi to detect bovine adenovirus antigen in pathological materials, the assay was repeated with faecal material spiked with bovine adenoviruses (serotypes 3 and 5) provided by V. Pálfi, Hungary. The faeces were tested both prior to and after spiking.

Results

Microbiological findings

The fungi, bacteria and viruses findings are summarized in Table 1.

No other bacteria including *Mycoplasma* spp. and *Chlamydia* spp. were found.

Adenovirus detection

All the samples from pathological cases which included eight faecal samples, two intra-articular fluids, and one aqueous

Table 1. Microbiological findings in diarrhoeic faeces taken from calves between 1 to 10 days old with weak calf syndrome symptoms

Rotav.	Cry.	S.E.	S.C1	C.g	C.c	G.c
3/8	2/5	3/7	3/7	3/8	5/7	3/7

Rotav, *rotavirus*; *Cry*, *Cryptosporidium parvum*; *S.E*, *Salmonella* (anatum); *S.C1*, *Salmonella hadar*; *C.g*, *Candida glabrata*; *C.c*, *Candia canulata*; *G.c*, *Geotrichium candidum*; Pos + /number of faeces samples available for testing.

humor, reacted by demonstrating a specific precipitate line as did the reference adenoviruses, while ten faecal samples from healthy neonates and ten diarrhoeic samples not related to this outbreak did not show this line.

Discussion

This report describes the first confirmation of adenovirus involvement in an episode of neonatal calf diarrhoea that resembled the weak calf syndrome noted elsewhere (Cutlip and McClurkin, 1975; McClurkin and Coria, 1975). Detection of bovine adenovirus was by a commercial kit (Rida® Quick rotavirus/adenovirus-combi) geared for human adenovirus diagnosis. This kit enables virus detection in few minutes.

The presence of adenovirus in the intestine as well as in the synovial fluid and aqueous humour in contrast to negative results from control animals strengthens our primary suspicion of this episode resembling the WCS published elsewhere (Cutlip and McClurkin, 1975; McClurkin and Coria, 1975; Stauber et al., 1975).

Some authors have suggested that endothelial cell damage in small caplliaries may be the initial lesion leading to ischaemia and increased vascular permeability resulting in diffuse haemorrhages at anatomical sites where there is a well developed vessel-bed, such as the intestines, joints and eye (Bulmer et al., 1975; Tùry et al., 1978).

Canadian authors reported that diarrhoeic calves infected with adenovirus were also heavily infected with fungi that they considered being a secondary infection in immuno compromised calves (Bulmer et al., 1975). Under certain management regimes, the presence of yeasts in the gastrointestinal tract might be associated with NCD (Elad et al., 1998). In a clinical controlled trial, Israeli authors found that dam's milk might exert a favourable effect on diarrhoeic calves with intestinal candidiasis (Elad et al., 2002). They concluded that dam's milk is able to reduce adhesion of *C. glabrata* to epithelial cells when compared with milk substitutes.

The presence of corona virus, *Coccidia and ETEC* (K99 + / F5) was also assessed but these enteropathogens were not detected.

No rapid procedure for the identification of bovine adenovirus exists in veterinary medicine, but the Rida® Quick rotavirus/adenovirus-combi test that is geared for rapid diagnosis in cases of human young diarrhoea seems to be a satisfactory solution.

In this case, recommendations included the use of dry straw for bedding from the first day of life in the neonatal parlour and in the outdoor pens. In addition, feeding the young calves whole milk for at least 14 days was advised, as it was the refraining from use of milk substitutes for the first 14 days of life. Prophylactic antibiotic therapy was discontinued.

After the above changes in husbandry were initiated, no additional deaths were recorded, and there was remarkable clinical improvement, especially in the reduction of joint swelling.

Currently, rapid commercial kits for the diagnosis of intestinal bovine adenoviruses are not available in most veterinary diagnostic laboratories as they are in human medicine. The NCD complex is a challenging problem with many unknown pathogenic factors involved, and any additional diagnostic information is extremely important.

At this time no vaccines are available for the prevention of this form of wasting calf syndrome.

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