

The Presence and Possible Effects of Bluetongue Virus in Goat Herds in Israel

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ABSTRACT

In spite of the many reports on bluetongue (BT) in cattle and sheep, publications on BT-attributed manifestations in goats naturally infected with bluetongue virus (BTV) are scarce, and up to now there have been no reports on BT-attributed manifestations in goats in Israel. In this retrospective study, which was carried out on 8 goat farms in Israel, high levels of neonatal morbidity or mortality, as well as abortions, were observed in goats which were concurrently infected with four different BTV serotypes (BTV-2, BTV-4, BTV-8, and BTV-16). It is not however clear whether BTV was the primary cause of disease in the goats, or whether it may have contributed as a secondary pathogen in predisposing the goats to disease caused by other pathogens.

Key words: Blue tongue virus, goats, clinical manifestations, disease, Israel

INTRODUCTION

Bluetongue virus (BTV) is the type species of the genus *Orbivirus*, within the family *Reoviridae*. Currently, 26 different serotypes of BTV have been identified according to the specificity of interactions between neutralizing antibodies and the virus outer capsid, the VP-2 protein (1, 2). BTVs are transmitted by the adult midges of certain *Culicoides* species and can infect all ruminants (3, 4). Clinical signs of BTV infection are usually confined to sheep, which may develop a severe hemorrhagic disease called bluetongue (BT). Elbers *et al.* (5, 6, 7, 8), introduced the concept of BT-attributed clinical signs during their study of the epidemiology of BTV infection across Europe. These signs included fever, anorexia, dysphagia, ulcerative and necrotic lesions of the oral mucosa, hyperemia and edema of the conjunctival mucosa, painful

muzzle, hyperemia of the teats and udder, hemorrhage, dehydration and lameness (9, 10, 11, 12, 13).

From 2008 to the present time (April 2012), eight BTV serotypes (BTV-2, BTV-4, BTV-5, BTV-8, BTV-12, BTV-15, BTV-16 and BTV-24) have been identified to be circulating in diseased ruminants in Israel (14). During this period, different BT syndromes were observed in Israeli cattle populations, including the 'footrot-like syndrome', 'sore nose syndrome', 'subcutaneous emphysema syndrome', 'red/rough udder syndrome', bluetongue/epizootic hemorrhagic disease systemic syndrome' and 'maladjustment syndrome' (14, 15, personal communications).

Although BT has a 70-year history in Israel (16), BT-attributed clinical signs have historically mainly been reported in sheep, with only sporadic cases of hemorrhagic disease

(HD), associated with unidentified viral agents, observed in cattle (17).

Publications on BT-attributed clinical manifestations in naturally infected goats are rare (18, 19). Currently, the general opinion among entomologists is that, although *Culicoides* species prefer to feed off cattle, all ruminant species, including goats are at risk from attack by infected midges. The clinical manifestations in goats exposed to BTV-infected midges are less prominent than the BT-attributed manifestations reported in dairy cattle and sheep herds (4). Although there have been reports that address BT-attributed manifestations in sheep and cattle in Israel, there have been no such publications on BT-attributed manifestations in goats (14, 15, 16).

This manuscript describes the levels of clinical disease seen in 8 goat farms over a 3-year period, and reports on the possible causal agents that were identified in the affected goats.

MATERIALS AND METHODS

Animals and farms

The locations of the BTV affected goat herds in this study are shown in Figure 1. No clinical data were collected 'in real time', as no clinical signs, even if presented at the time, were attributed to BTV infection, and the disease incidents were not reported to veterinary officers. BTV was initially found in the carcasses of goat kids sent to the Kimron Veterinary Institute (KVI), which were tested for BTV as a result of increased awareness of BTV by the arbovirus laboratory staff. Consequently, further retrospective investigations, based on case histories obtained from the farmers and from the local veterinary practitioners, were carried out on eight dairy-goat farms, and the clinical data were recorded.

Sample collection and laboratory testing for BTV

Blood samples (EDTA and whole blood) and internal organs from goats showing clinical signs, such as unexplained abortions, respiratory distress, unusual morbidity and/or mortality were initially BTV-PCR tested at the KVI (14). Samples tested BTV-PCR positive were subsequently sent to the Institute for Animal Health, Pirbright, UK (IAH-P), where they were tested by group-specific and type-specific RT-PCR to confirm the presence of BTV and to identify the BTV serotype. Further clinical data were collected retrospec-



Figure 1: Geographic location of the dairy-goat farms infected with bluetongue virus.

tively only in cases in which BTV was diagnosed either by PCR or by virus isolation.

Differential diagnosis – laboratory testing

Samples from clinical cases received for testing at the KVI were routinely tested for an array of pathogens compatible with the clinical signs reported on the farms. Samples from animals that had presented with abortions or genital manifestations were routinely tested for brucella, Q fever, toxoplasma, *Neospora* spp., border disease (BD), and *leptospira* (eight serovars). In some cases toxicological tests were applied, for example, nitrate substance levels in the eye of dams which had aborted. Tests for neonatal kid diseases were performed in accordance with the reported symptoms: samples from animals with 'watery mouth syndrome' were tested for systemic *E. coli* infection, whereas those associated with respiratory distress were tested for respiratory syncytial virus (RSV) and general bacteriology was carried out, with particular attention to *Mycoplasma ovipneumonea* (20). Yearling kids' samples were tested for enterotoxemia (pulpy kidney disease).

RESULTS

Overall BTV findings in the eight goat farms

During the investigations the presence of BTV RNA was confirmed by RT-PCR in 8 dairy-goat herds in Israel. The serotypes found in goats were: BTV-2, BTV-4, BTV-8, BTV-16, and in one case the serotype was not identified. Most of the affected farms, reported both morbidity and mortality in young kids, but some farms reported sporadic clinical manifestations in adult goats.

Farm A

Farm A housed an intensively managed mixed-breed dairy goat herd and was located in the hills of the Upper Galilee. In December 2008, an acute respiratory outbreak affected almost all of the 600 kids aged between 4 and 5 months that were present on the farm. The clinical manifestations, reported by the owner, included nasal discharge, fever (above 40°C), and coughing. Antibiotic treatment did not prevent mortality, and 40 yearling kids died after 3-4 days of severe respiratory distress: four of them were sent to the KVI for post mortem examination and microbiological laboratory investigation. The laboratory test results related to the 2008-2009 episodes are shown in Table 1. The negative serological results, along with the positive results obtained by BTV-PCR, indicated that the goats were in the early stage of infection, before antibodies to BTV had developed (about 3-8 days post infection).

In November 2010 a single adult goat was presented from the farm with a high temperature, scialorrea and was recumbent. No BD or RSV was detected and the only confirmed pathogen found was bluetongue virus serotype 8 (BTV-8).

Farm B

Farm B was a small backyard holding of about 20 small ruminants, located in the central Coastal plain of Israel (near the town of Yavne). In mid-November 2010 two does delivered dead kids at normal pregnancy term, and a few days later a bloody fluid discharge was observed oozing from the vagina of both does. Following negative results for all abortive pathogens – Q fever, *Leptospira* spp., (8 serovars) *Chlamydia* spp., *Toxoplasma*, *Brucella*, and *Pestivirus* (border disease (BD)) – one goat was found BTV seropositive, and BTV-2 was confirmed to be present in the EDTA blood sample taken from the goat. No other BT-attributed clinical manifestations were observed among the adult group of 12 pregnant goats and sheep housed on the farm.

Farm C

Farm C was located 60 km north of the Red Sea in a harsh dry region with less than 40 mm annual rainfall. In April 2010 this dairy-goat farm reported high levels of mortality and the births of weak kids. The does remained apparently normal. Five blood samples that were sent to the KVI were found BTV antibody positive and all other abortive agents were ruled out. Of the 60 kids that were born, half were reluctant to suckle colostrum and remained recumbent, and some of them did not seek shade. Twenty of them died before reaching 10 days of age (case mortality rate of 66.7%).

Farm D

Farm D was located in the coastal plain at the foot of the Samarian hills. In November 2010 the owner reported a severe respiratory outbreak that was not responsive to broad-

Table 1: Summary of the laboratory findings during the 2008-2009 BT-outbreak in Farm A:

Kid	*PCR-BTV-16	BTV Ab ELISA	RSV-Ag**	<i>In-ovo</i> isolation (% egg mortality)	Bacteriology
1	positive	negative	negative	100% mortality	Mixed culture
2	positive	negative	positive	100% mortality	Mixed culture
3	positive	negative	positive	100% mortality	<i>C. perfringenes</i> & <i>P. aeruginosa</i>
4	not done	negative	negative	not done	Mixed culture
Total	3/3	0/3	2/3	3/3	1/4

* BTV-16 was identified in IAHP, Pirbright, UK;

** Respiratory Syncytial Virus Ag (in lungs) found with RSV DFA Kit (Light Diagnostic, Temecula CA, USA): *C. Clostridium perfringenes* D & *P. Pseudomonas aeruginosa*

spectrum antibiotic treatment. A total of 34 goats died: 17 out of 100 pregnant does between 8 and 10 months old, 8 out of 220 older milking does, and 9 out of 100 younger kids. According to the owners all the diseased animals died following 7-10 days of clinical signs, bringing the case mortality to 100%.

One pregnant doe was sent to the KVI for pathological and microbiological examination. Bacterial and mycoplasma test findings were negative, and virological tests, including for FMDV, RSV, para-influenza virus-3, PPR, and BD, were also negative. The lung and spleen from the goat tested positive by RT-PCR for Bluetongue virus serotype 4 (BTV-4).

The post mortem findings revealed a characteristic subacute chronic pneumonia, diffuse hyperemia in all lobules, with well developed lung parenchyma consolidation of 90% of the lung mass. No pathological changes were noted in the abdominal cavity but a 3-cm-long embryo was found in the uterus. Histological examination revealed alveolar and interlobular edema, hyperplasia of the bronchial epithelia, prominent augmentation of the volume of the broncho-associated-lymphatic tissues, and histiocytic and neutrophilic infiltration.

Adjacent cattle and sheep herds had been diagnosed with BTV infection at the same time as the occurrence of disease in the goat farm. Moreover, during the 2008-2011 BT outbreaks, BTV-4 was found in some sheep herds, where severe symptoms were observed (personal communication).

Farm E

Farm E housed a herd of dairy goats (Za'anen), comprising about 400 nannies was located near the Lebanese border, in a zone infected with BTV-4, BTV-8, and BTV-16 (14, 15). The farm suffered a loss of 250 kids and almost 100% of all the 250 neonates died during the labor season (February-April) of 2010, all the kids dying before reaching 72-96 hours of age.

Because of the early-neonatal mortality and massive scialorrhoea observed in the affected neonates, FMD was initially suspected but ruled out. Watery-mouth disease (*Escherichia coli* infection) was then suspected and hemolytic *Escherichia coli* were isolated from the internal organs (liver and spleen). *Manheimia haemolytica* were isolated from the lung and BTV-8 was isolated on cell culture from the spleen of this kid and confirmed by IAH-P. No other pathogens were identified in the neonate, which also presented with severe necrotic pneumonia.

Farm F

Farm F was located near the Mediterranean coast in the Akko Gulf. During February-March 2011 the farm reported abortions in one yard that housed 80 pregnant animals, both pluri- and primiparous. In eleven does, abortions occurred during the 3rd trimester of pregnancy, and an additional seven goats died, bringing the total number of affected goats to 18. On this farm, which housed 400 animals, diseased goats were noted initially only in the pregnant group, and mortality of does was recorded prior to or immediately after parturition. This dairy farm also contained dairy sheep but they remained unaffected.

This herd of goats was acquired from several different sources, and the owners were unable to identify the original site(s) from which the diseased animals originated. The appearance of the clinical manifestations in less than one week from their arrival to the farm, suggested that the goats may have been already infected with the causal agent prior to being introduced on the farm.

Tests for abortive agents including Q fever, *Leptospira* spp., (eight serovars), *Chlamydia* spp., *Toxoplasma*, *Brucella*, proved negative. Three out of five blood samples that were sent to the KVI tested BTV positive by RT-PCR. The BTV reference laboratory at the IAH-P confirmed one of the samples to be BTV-8- positive.

During the first 10 days of June 2011, all the affected group of 80 does delivered kids that presented signs compatible with the weak-kid syndrome (21). The same occurred in an additional local group of 60 goats that did not present any clinical manifestation. Fifty out of 140 newborn kids died before reaching 7 days of age.

Five of the kids sent to the KVI presented with profuse watery diarrhea: major pathological changes were noted in the small intestine, where small hemorrhages and reddish/yellow fluids were found. *Cryptosporidium* was identified in all the kids, and *Salmonella group E* was isolated from two of them.

Farm G

Farm G was an agricultural high school near Ashkelon that housed about 100 milking goats. In January-March 2010 an episode of kid mortality was reported and the entire litter of 80 kids presented with weak-kids syndrome. Nevertheless, no clinical signs were noted in the adult goats. Twenty kids died before reaching 1 week of age (25% mortality rate), and the immediate suspicion was of toxemia/toxicosis. No py-



Figure 2: Double sole in a goat infected with BTV-16.

retheroides, organo-phosphateic, carbamate, or chloral compounds were found, but one kid out of eight tested positive for *Clostridium perfringens* type D. BTV-16 was isolated on cell culture from the spleen of one of the kids that was sent for viral diagnosis at the KVI laboratory. BTV-16 was confirmed to be present in the sample at the reference laboratory at IAH-P. The attending personnel at the farm reported stiff gait among the adult goats on the farm and many of the goats exhibited a "double-hoof phenomena" (Figure 2), i.e., a demarcation line between the new and old tissue, which could support a link between BTV-16 exposure and the reported stiff gait. No other relevant bacterial or viral agents such as BD were identified in the goats.

Farm H

Farm H was a small zoo in the southern portion of the Carmel Mount. Twenty goats on the farm exhibited characteristic lesion of *ecthyma contagiosum* during December 2011. ORF virus was identified by a routine PCR procedure in the viral diagnostic laboratory of the KVI. Whole blood in EDTA, taken from the infected animals, was also positive for BTV-8. Four of the six animals were found to be infected with both ORF and BTV and the remaining 2 samples were BTV positive but ORF negative.

DISCUSSION

Severe outbreaks of disease have been observed and reported in various goat herds across Israel which was concurrently in-

fectured with four different BTV serotypes (BTV-2, BTV-4, BTV-8, and BTV-16). It is not however clear whether BTV was the primary cause of disease in the goats, or whether it may have contributed as a secondary pathogen in predisposing the goats to disease caused by other pathogens. Alternatively it is possible that BTV may be circulating in the livestock and goat herds in Israel without causing clinical manifestations. It is however clear that many of the diseased goats that were sampled in this study tested positive for BTV, so it is possible that BTV may have played a direct or indirect role in disease progression and pathogenesis in the goat populations of Israel.

Backx *et al.* 2007 (18) challenged goats with the European BTV-8 strain and obtained mild clinical signs, whereas all inoculated sheep became severely ill. In another experiment, the use of raw material obtained directly from a naturally field-infected sheep induced clinical signs in goats, possibly due to the fact that the challenge virus had not been attenuated through tissue culture passage prior to the challenge (18). Although clinical signs have been observed in goats after experimental infection (18), there have been few cases of clinical disease observed post natural field-acquired infection. Moreover, even the new Toggenburg orbivirus (TOV) did not induce BT-related clinical signs in adult goats following experimental challenge (22). Chaignat *et al.* 2010 found that in a large TOV serosurvey carried out in Switzerland only one goat was reported to have shown BT-like symptoms (23).

The present study reports a high prevalence of clinical manifestations in neonates, in contrast to small number of adult animal showing clinical signs. In four of the farms (Farms A, E, F and H) examined, BTV-8 was identified, but no clinical signs, apart from the one single case in Farm A, were reported in adult animals. In Farm F no enteric or respiratory signs were detected in neonatal or young kids, whereas in Farm A, *E. coli* sepsis was identified which was the probable cause of the 'watery mouth syndrome'. However, the question of whether the excessive high neonatal mortality was associated with BTV-8 infections remains unanswered and warrants further investigation. Only two farms (Farms D and F) reported sudden mortality in adult goats (without showing BT-attributed clinical signs prior to their death), however it is possible that these deaths were BT-related. In Farm A, a relatively high mortality was reported in the yearling group of goats, which may have been caused by a

dual infection with BTV-16 and respiratory syncytial virus (RSV), the latter of which has previously been associated with outbreaks of respiratory disease in goats (24). The negative serological results in the sera of the goats from Farm A, along with the positive results obtained by BTV-PCR, indicated that the infected animals were in the very early stage of infection with BTV. This active infection with BTV may have contributed to the severe respiratory signs observed, which resulted in the death of so many young goats on the farm. The fact that the adult goats on the farm did not show any clinical signs consistent with BTV infection indicates that they were either clinically resistant to BTV or they had been previously exposed to and were immune to BTV-16.

This study revealed a relatively high level of abortion in adult goats combined with high levels of neonatal mortality in kids, both of which interestingly coincided with the relatively recent introduction of the European strain of BTV-8 and the first identification of clinical disease in cattle attributed to BTV in Israel, in around 2008. It is possible that the increased levels of circulation of BTV serotypes in Israel (14), including the circulation of the European strain of BTV-8 which is known to cross the placenta and infect offspring, is either directly or indirectly contributing to the increased levels of reproductive disease observed (25). The observation that BTV infection might lead to abortions in white-tailed deer was suggested as far back as 1970 (26) and this finding may add evidence to the role of BTV in abortions among does as documented in this study in Israel.

This manuscript reports the presence of 4 serotypes of BTV in goats, in Israel. Adult goats from farms where BTV was identified to be circulating showed mild and sporadic clinical signs, however, high levels of kid-mortality and suspected abortions were observed on the farms. However, further more detailed studies are required to confirm whether BTV is playing a primary, a secondary or no role at all in the high level of disease observed on the goat farms in Israel.

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