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Vaginal Enterobius Vermicularis Diagnosed on Liquid-Based Cytology During Papanicolaou Test Cervical Cancer Screening: a Report of Two Cases and a Review of the Literature.

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3 **Vaginal Enterobius Vermicularis Diagnosed on Liquid-Based Cytology During Papanicolaou**
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6 **Test Cervical Cancer Screening: a Report of Two Cases and a**
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8 **Review of the Literature.**
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13 **Running title:** Enterobius vermicularis on Pap Test.
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Abstract

Enterobiasis is one of the most common human parasitic infections. It is considered an intestinal parasite, but cases of extra-intestinal affections exist, notably infections of the female genital tract. *Enterobius Vermicularis* eggs (or ova) have been found in the cervical smears of two patients in our institute during the last sixteen years. No gynaecological or gastrointestinal symptoms were reported, and there was no known intestinal infection in these two cases. A review of the available literature revealed rare cases of vaginal enterobiasis, with a wide range of clinical presentations, many patients being asymptomatic. The diagnosis may sometimes be difficult, mainly because of the lack of clinical suspicion. However, cytological identification of *Enterobius Vermicularis* in cervico-vaginal smears is important, especially when considering the risk of ascending infections of the genital tract associated with severe complications.

Keywords: Gynecologic cytology, Papanicolaou test, Liquid-based cytology, Vaginal *Enterobius Vermicularis*.

Introduction

Enterobiasis is the most frequent parasitic infection in humans, with a prevalence of 35% to 70%.¹ It is more common in temperate countries (United States and Western Europe).^{2,3} Biologically speaking, *Enterobius vermicularis* (EV), also called the human pinworm, is a helminth (parasitic worm), belonging to the class of nematodes. Humans are the only known host of EV. The transmission occurs by anus-to-mouth transfer by contaminated hands, directly or indirectly via contaminated surfaces, or with the ingestion of the infective eggs (or ova).³ Self-infection as well as person-to-person transmission may occur. Spread can also occur via airborne transmission of aerosolized eggs. Once ingested, the larvae contained inside the eggs are released in the small intestine, and the adult worms diffuse to the caecum and colon. Gravid females then migrate at night to the perianal region and to the perineum where they lay eggs.²⁻⁴ Clinical presentation of intestinal enterobiasis is variable, with many patients being asymptomatic. Frequently described symptoms comprise perianal pruritus and secondary excoriations, sometimes complicated by bacterial superinfection. Anorexia and abdominal pain are other possible symptoms.²

Infrequently, EV may enter the female genital tract.² A proposed mechanism of genital infection is migration of gravid female worms from the perianal region to the vagina. From there, they may ascend to the uterine cavity, and even reach the peritoneum through the fallopian tubes. The fact that only female worms and eggs have been found in cervical samples supports this theory.³ Vaginal enterobiasis has variable clinical presentations. It may manifest with vaginitis, vulval pruritus and vaginal discharge, but most reported cases of vaginal enterobiasis (> 70%) were asymptomatic.⁵ Adequate treatment of vaginal pinworm infections is

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3 paramount because of the possible involvement of further structures such as the fallopian
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6 tubes or ovaries, with the risk of severe complications like bacterial co-infection with bleeding,
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8 infertility, tubo-ovarian abscesses and generalized peritonitis.^{3,6-8} Thus, the Papanicolaou (Pap)
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10 test has become an important diagnostic tool in the detection of such infections.
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14 The presence of EV in a liquid-based cytology (LBC) sample during a cytological
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16 examination performed for cervical cancer screening is quite challenging as it poses a
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18 differential diagnosis between a true vaginal infection following migration of the worm from
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20 the digestive tract versus a contamination of the samples with eggs coming from the perineum.
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24 Subsequent to finding EV eggs in the cervical smear of one patient at our institute, we
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26 searched through our files for other cases. Here we present two cases of pinworm eggs found
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28 in routine Pap tests examined since the introduction of LBC for cervical cancer screening,
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30 between 2001 and 2017. We also undertook a complete review of the literature on EV
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32 diagnosed in cervico-vaginal smears.
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Case reports

Case 1: A 43 year-old G2P2 female, suffering from rheumatoid arthritis, currently under immunosuppressive treatment (Actemra), underwent a routine cervical smear. Clinical information provided in the cytological exam sheet read: "annual control". The cervical smear, processed with the Thin Prep technology (Hologic Inc, Boston, Massachusetts, USA), showed predominantly superficial cells without significant inflammatory changes. Amidst them, approximately a dozen EV eggs were seen, some containing granular material, some empty, associated with a few embryos (Figs. 1A-1E). All the possible spectrum of association between eggs and embryos has been observed: some embryos were entirely inside the eggs (Fig. 1A), some were partially expelled (Figs. 1B-1D) and some completely expelled without any contact with the eggs (Fig. 1E). A few epithelial cells were diagnostic of low-grade intraepithelial lesion (LSIL) (Fig. 1F). The cytology report concluded with "LSIL and presence of pinworm eggs". According to the available medical records that were consulted after the diagnosis, the patient had a medical history of two spontaneous vaginal deliveries and HPV infection 15 years earlier, with a biopsy diagnosis of cervical low grade squamous intraepithelial lesions (LSIL) as well as low grade vaginal intraepithelial neoplasia (VaIN1). She was originally treated with laser and presented with repeated abnormal follow up cervical biopsies (LSIL and VaIN1) after initial treatment. The colposcopic examination performed during the actual Pap test was unremarkable (Table I).

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4 Case 2: A 17 year-old female had a prenatal cervical cancer screening during early
5 pregnancy. Similarly to the other patients, clinical information provided in the cytological exam
6 sheet read: “annual control, pregnancy”. The Pap test slide demonstrated superficial cells
7 admixed and partially covered by inflammatory cells, mostly neutrophils and eosinophils (Fig.
8 2A). Only one EV embryonated egg, containing a coiled larva that was partially delivered, was
9 identified (Fig. 2B). Some squamous cells showed slight nuclear enlargement. The cytological
10 diagnosis was “Atypical squamous cells of undetermined significance (ASC-US), severe
11 inflammation with reactive cellular changes; presence of one pinworm egg and embryos”. As
12 the Pap test was performed in the context of a family planning program more than fifteen years
13 previously, no additional data about the patient’s medical history follow up or treatment was
14 available (Table I).
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Discussion

Despite the fact that EV is an intestinal parasite, rare cases of extra-intestinal infections, mostly involving the female genital tract, have been reported.³ As stated previously, the fertilized female worms normally migrate from the colon to the perianal area or the perineum where they deposit their eggs. Rarely, they may reach the genital organs of women. The frequency of involvement of the genital tract is unknown because only scarce reports of extra-intestinal enterobiasis, in the ovary, vagina, salpinx, and peritoneal cavity can be found in the available literature.⁹⁻¹¹ Concerning findings of EV in the vagina reported throughout the world, the literature in English contains approximately thirty cases of EV, mostly EV eggs, discovered in vaginal or cervical smears, since the 1970s (Table II).^{1,5,11-30} Interestingly, the majority of the cases reported come from India, even though enterobiasis seems more prevalent in temperate than tropical countries.² In some of the reported cases there were no details about the specimen processing methods (conventional smear or liquid-based cytology). It must be mentioned that we found five more papers focused on vaginal enterobiasis that appeared between 1970 and 1986 in France, Poland, Croatia, Italy and Turkey, published only in their original language.³¹⁻³⁵ At our Institution, the two cases described here are the only ones among the 166,976 cervical smears that were examined during the last 16 years, thus representing a very small percentage (0,001%).

Most cases reported in the literature are fortuitous diagnoses, and reflect the variability of clinical presentation. Because a significant proportion of patients are symptomatic, thus raising suspicions of other infectious diseases or even malignancies, a correct diagnosis is important. In a case reported in 2015 in a 40-year-old woman presenting with vaginal discharge, clinical

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3 examination of the cervix showed its aspect to be strongly suspicious of cervical cancer.
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6 However, the Pap smear showed a few EV eggs containing larvae, and bacterial vaginitis. The
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8 patient was treated with antibiotics and anthelmintics, with a good response.¹
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11 The number of eggs discovered in the reported cases varies greatly: Wong described
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13 four cases of vaginal enterobiasis in Texas, with one of the smears containing only 2 eggs and
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15 another one containing more than 400 eggs.²² Rad reported one case in Canada, in which
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17 approximately 2000 eggs were found in an endocervical smear.²⁸ When described, the smears
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19 often show an inflammatory background. Langlinais reported 1 case in the USA, with a vaginal
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21 smear containing numerous eggs and a worm among many polymorphonuclear leukocytes, as
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23 well as *Trichomonas*.²⁹ Mali described 6 cases in India: the slides from two of them showed
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25 severe inflammation, with cellular debris, whereas the four other cases revealed no signs of
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27 significant associated inflammation.¹⁷ Avram reported four cases in Israel, but only one slide
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29 was described, in which eggs were observed on a mild inflammatory background.¹⁹ In India, an
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31 unusual case of a 4-year-old girl was reported, who presented recurrent vaginal enterobiasis
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33 despite treatment (anthelmintic *mebendazole* over a period of 3 months was given to the
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35 patient and her family, on all occasions). The patient complained of repeated episodes of vulval
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37 itching and clinical examination revealed an inflamed vulva with 4-5 living worms. Every time,
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39 direct microscopic examination of vaginal swabs revealed adult worms of EV without eggs.
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41 There were no gastrointestinal symptoms, and repeated stool samples of the patient and her
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43 family were negative. In this case, recurrent vaginal infection by EV despite treatment and in
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45 the absence of any signs of gastrointestinal infection suggests that the vagina is a potential
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47 reservoir.¹²
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3 The ability of EV to survive and reproduce in the female genital tract has been discussed
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5 previously, with the hypothesis that the eggs can hatch and the larvae can survive in the moist
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7 environment of the vagina.³² Moreover, this case emphasizes the lack of experience and data
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9 about efficient treatments for vaginal enterobiasis.¹²
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12 Concerning diagnosis, cytological features of EV eggs are quite specific. Eggs are ovoid,
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14 flattened on one side, measuring 50-60 μ in length and 20-30 μ in width.^{3,13} They have the
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16 appearance of pumpkin seeds, with a characteristic thick, double contoured birefringent shell.
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18 Some of them contain coarsely granular embryos or curved larvae in different delivery
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20 situations, like in our case 1. They may be difficult to identify due to the accompanying
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22 inflammation,⁵ as shown by our second reported case.
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29 When carrying out a differential diagnosis, the vaginal samples may be contaminated by
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31 eggs deposited in the perineal area, or there may be confusion with other parasitic eggs, pollen
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33 grains, contaminant vegetable cells, or fungal spores.^{1,13} A clue to the diagnosis of vaginal
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35 enterobiasis versus contamination is the presence of an important acute inflammatory
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37 background that should be found in the presence of parasitic infections.⁵ Parasitic infections
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39 can also be distinguished from contaminant vegetable cells and fungal spores by observing the
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41 characteristic refractile sheath containing bright orange-staining EV larvae, that is not found in
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43 vegetable material or fungi.^{24,36} Pollen grains can be identified as well. They consist of two
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45 layers containing grains, with an outer layer that may be smooth or rough because of the
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47 presence of warts, grains or troughs.¹⁴ The differential diagnosis also comprises other parasitic
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49 infections: *Entamoeba histolytica*, *Microfilaria*, *Strongyloides stercoralis*, *Schistosoma*
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51 *haematobium*, *Trichuris trichiura*, *Ascaris* and *Taenia*.¹ The diagnosis should be made with
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3 clinical correlation and investigations such as stool examination and culture. Microscopic
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6 distinction should be possible based on the different morphological characteristics of the
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9 parasites and of their eggs.

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11 In conclusion, even if rare, EV eggs, embryos and larvae can be found in cervical Pap
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13 smears of asymptomatic woman during routine controls for cervical cancer screening and
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16 should not be missed or confused with pollen, vegetable material or fungal spores.
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3 **Figure legends**
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8 **Fig.1.** Eggs of EV containing embryos that are either entirely inside the egg (1A), partially
9 expelled (1B-1D) or completely delivered (1E). Epithelial cells with features of low-grade
10 intraepithelial lesion (LSIL) (1F)(Papanicolaou staining, 600x).
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16 **Fig.2.** Superficial cells largely covered with neutrophils and eosinophils (2A)(Papanicolaou
17 staining, 400x). One EV egg (2B, asterisk) containing a coiled larva that is partially delivered (2B,
18 arrow head)(Papanicolaou staining, 200x).
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Table I. Clinicopathological data of the two patients presented.

| Case n.° | Age | Context | Symptoms, clinical history | Specimen type | Microscopic features of EV | Slide background | Associated squamous lesions |
|----------|-----|-------------------------------------|---|-----------------|--|--|--|
| 1 | 43 | Routine screening. | No clinical symptoms. Past medical history: G2P2; Rheumatoid arthritis with immunosuppressive treatment. | Cervical smear. | A few EV eggs: some of them contained granular material, some others were associated with embryos. | No significant inflammation | Features of LSIL in a few epithelial cells |
| 2 | 17 | Routine screening, early pregnancy. | NR. | Cervical smear. | One EV egg containing a partially delivered larva. | Severe inflammation (presence of neutrophils and eosinophils), with reactive cellular changes. | A few ASC-US. |

EV = Enterobius Vermicularis; LSIL = Low-grade squamous intraepithelial lesion; NR = Not reported; ASC-US = Atypical squamous cells of undetermined significance

Table II. Clinicopathological data of patients (n= 76) diagnosed with EV in Pap Test from cases reported in the English literature.

| Case n.° | Author, year, reference | N.° of reported patients | Study type; country of origin | Age | Symptoms | Clinical examination | Specimen type | Microscopy | Follow up |
|----------|------------------------------------|--------------------------|--|------------------------|--|--|--|--|---|
| 1 | Raju, 2015 ¹ | 1 | Single case report; India. | 40 | Vaginal discharge; no symptoms of intestinal oxyuriasis. | Hypertrophied cervix with eversion of both lips suspicious for cancer. | Cervical smear | Background of neutrophils and coccobacilli; 2-3 eggs of EV containing coiled larvae. | Treatment with antibiotics and antihelmintics; good response. |
| 2 | Kashyap, 2014 ¹² | 1 | Single case report; India. In this case, the vagina seems to be a potential reservoir for EV. | 4 | Recurrent episodes of vulval itching (over 2 years). | Vulva inflamed, with 4-5 living worms emerging from the anterior vaginal fornix, no discharge. | Vaginal swabs (repeated) | Adult worms of EV, without any eggs. | Despite complete treatment, adult worms were found in the vagina on multiple occasions. |
| 3 | Shetty, 2012 ¹³ | 1 | Single case report; India. | 35 | Vulvar pruritus and vaginal discharge | Excessive vaginal discharge. | Cervical and vaginal smears | Embryonated eggs of EV, containing coiled larva; numerous acute inflammatory cells in the background. | Treatment with antihelmintics. Patient symptom-free post treatment. |
| 4 | Seung-Kyu, 2010 ⁵ | 1 | Single case report; Korea | 37 | No clinical symptoms | NR | Cervicovaginal smear | Grouped or individually scattered EV eggs containing embryos or curved larvae; inflammatory background, no eosinophils or histiocytes. | NR |
| 5 | Martinez-Giron, 2007 ¹⁴ | 1 | Single case report; Spain. | 59 | Past medical history: breast carcinoma (diagnosed 1 year earlier). | NR | Cervicovaginal smear | Typical worm egg in an atrophic smear, containing embryonic material; clean background, without inflammation. | NR |
| 6 | Chung, 1997 ¹⁵ | 1 | Single case report; Korea | 33 | Postcoital spotting; flank pain; vaginal discharge. | Erosion of the cervix; one live worm seen in the posterior fornix of the vagina | Vaginal smear | EV eggs | NR |
| 7 | Pandit, 1989 ¹⁶ | 1 | Review of 65'935 cervical smears screened to rule out cancer during 6 years: 1 case with EV eggs (0,0015 %); India. | 22 | Amenorrhea during 6 months | The cervix appeared normal. | Cervical smear | Numerous EV eggs containing larvae. | NR |
| 8 | Mali, 1987 ¹⁷ | 6 | 6 cases with EV eggs among 57'664 smears screened for cancer, recorded between 1971 and 1984 (0,01 %); India. | NR | Asymptomatic | NR | Cervical smears and smears from contraceptive devices. | Eggs of EV containing larvae; 2 cases with severe inflammation; 4 cases with negative pattern. | NR |
| 9 | Bhambhani, 1985 ¹⁸ | 7 | 7 cases report from smears for screening for preneoplastic/neoplastic/inflammatory lesions; India. | Reproductive age group | Vaginal discharge | NR | Cervicovaginal smears | 6 cases with single isolated eggs; 1 case with numerous eggs in a focal area. | NR |
| 10 | Avram, 1983 ¹⁹ | 4 | Mass screening program for detection of cervical cancer between 1978 and 1982: 2 cases of EV eggs among 22'700 cervicovaginal smears (0,0088 %); Israel. | 6 to 23. | NR | NR | Vaginal smears | Mild inflammatory background. | NR |
| 11 | Bak, 1982 ²⁰ | 12 | Investigation about the incidence of enterobiasis in 55'600 vaginal smears from routine cervical cancer detection program: 12 cases (0.02 %) of vaginal EV; Hungary. | 19 to 53 | Vaginal discharge | NR | Vaginal smears | EV eggs; 1 gravid female worm in 1 case; some cases showed many neutrophils; presence of Trichomonas in 3 cases, Candida Albicans in 2 cases. | NR |
| 12 | Saraswathi, 1982 ²¹ | 1 | Single case report; India. | 18 | Vaginal discharge. | NR | Vaginal wet smear | Eggs of EV, with a larva hatching out of the egg. | NR |
| 13 | Wong, 1981 ²² | 4 | 4 cases of EV eggs found among 220'000 routine cervicovaginal smears examined between 1971 and 1980 (0,0018 %); Texas (USA). | 23 to 30 | No symptoms reported. | NR | Cervicovaginal smears | Embryonated eggs in different stages of maturation; inflammation (neutrophils, but no eosinophils), reactive and reparative columnar cells, slightly atypical metaplastic cells. | No pinworm eggs found in subsequent smears from these 4 patients. |

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|----|-----------|-----------------------------------|----|--|----------|---------------------------------------|---|--|---|--|
| 1 | 14 | Bhaskaran, 1980 ²³ | 1 | Context of mass screening: 1 case of EV infestation of the cervix among 1100 routine cervical smears examined in 1978 (0,01 %); India. | 45 | White vaginal discharge. | Atrophic vagina; small and anteverted uterus; erosion of posterior lip of the cervix. | Cervical smear. | Clusters of refractile, brown staining eggs, resembling EV eggs; inflammatory cells (neutrophils, eosinophiles, few lymphocytes); some amount of cytolysis. | Patient lost to follow up. |
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| 5 | | | | | | | | | | |
| 6 | 15 | Garud, 1980 ²⁴ | 2 | Single case report; India. | 20 | Patient with threatened abortion. | NR | Vaginal smears | One EV egg containing larvae; no excess inflammatory response, not many histiocytes. | NR |
| 7 | | | | | | | | | | |
| 8 | | | | | | | | | | |
| 9 | 16 | Moscolo, 1979 ²⁵ | 1 | Single case report, Italy. | 21 | Dyspareunia; leuko-xanthorrea | Direct examination of the vagina fluor showed eggs containing larvae. | Vaginal smear | Nearly 40 eggs; numerous neutrophils, superficial and intermediate cells, scarce columnar cells. | NR |
| 10 | | | | | | | | | | |
| 11 | | | | | | | | | | |
| 12 | 17 | San Cristobal, 1976 ²⁶ | 1 | Single case report; Spain. | 11 | Vulvar itching. | Vulvovaginitis with slight vaginal discharge | Vaginal wet smear | EV eggs containing embryo. | Vaginal smears were repeated and showed neither eggs nor larvae. |
| 13 | | | | | | | | | | |
| 14 | | | | | | | | | | |
| 15 | | | | | | | | | | |
| 16 | 18 | De Torres, 1973 ¹¹ | 13 | Routine cytology : 13 cases of EV in 22'638 vaginal smears (0,057 %); Mexico. | 14 to 50 | Most cases only had vaginal discharge | NR | Vaginal smears | EV eggs; no other lesions described. | NR |
| 17 | | | | | | | | | | |
| 18 | 19 | Shipton, 1973 ²⁷ | 1 | Single case report; Australia | 43 | NR | Normal multiparous cervix | Cervicovaginal smear | Numerous eggs of EV, no adult worms. | NR |
| 19 | | | | | | | | | | |
| 20 | 20 | Rad, 1970 ²⁸ | 1 | Single case report of EV in the cervical canal; Canada | 31 | NR | Leukorrhea and cervicitis. | Cervical scraping smear and endocervical aspiration smear. | Massive inflammatory exudate; approximately 2000 EV eggs with larvae; some inflammatory cells | Repeated smear 2 weeks later contained no egg. |
| 21 | | | | | | | | | | |
| 22 | 21 | Langlinais, 1969 ²⁹ | 1 | Single case report; USA | 18 | NR | NR | Vaginal smear | Numerous parasite eggs, containing larvae; adult female worm containing 88 eggs; many neutrophils; numerous Trichomonas. | No treatment reported. Smears taken 3 weeks later were negative. |
| 23 | | | | | | | | | | |
| 24 | | | | | | | | | | |
| 25 | | | | | | | | | | |
| 26 | 22 | Brady, 1947 ³⁰ | 14 | Study on 45 cases of known intestinal enterobiasis; USA | NR | NR | NR | Single swabs made at the introitus | EV eggs | NR |
| 27 | | | | | | | | | | |
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EV = Enterobius Vermicularis; NR = Not reported; IU = Intrauterine.

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Figure 1

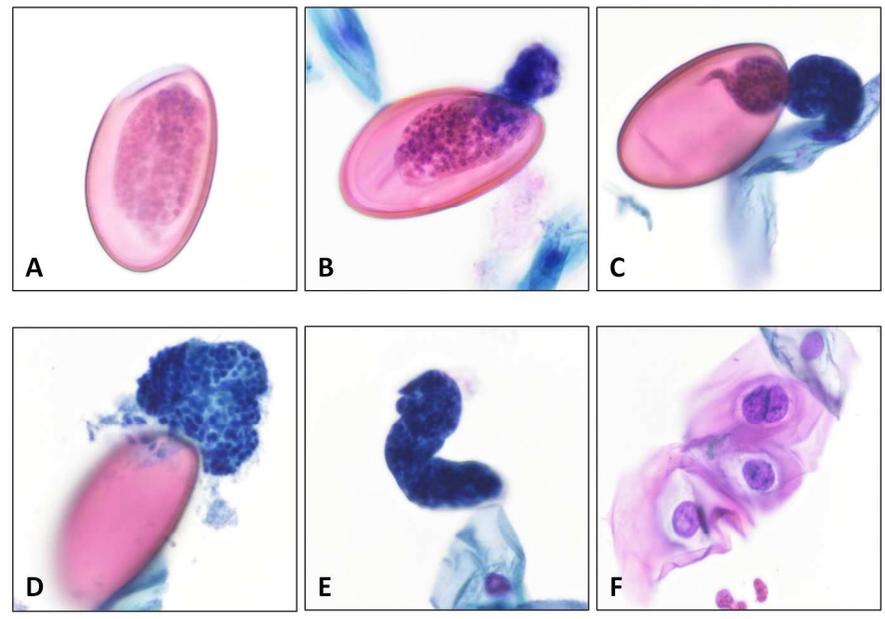


Fig.1. Eggs of EV containing embryos that are either entirely inside the egg (1A), partially expelled (1B-1D) or completely delivered (1E). Epithelial cells with features of low-grade intraepithelial lesion (LSIL) (1F)(Papanicolaou staining, 600x).

190x254mm (300 x 300 DPI)

Figure 2

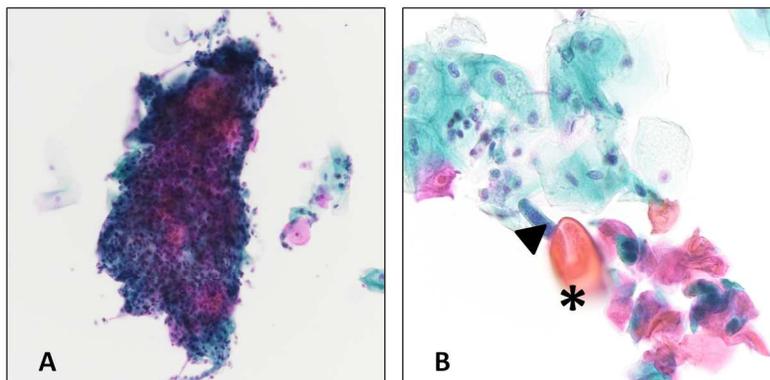


Fig.2. Superficial cells largely covered with neutrophils and eosinophils (2A)(Papanicolaou staining, 400x). One EV egg (2B, asterisk) containing a coiled larva that is partially delivered (2B, arrow head)(Papanicolaou staining, 200x).

190x254mm (300 x 300 DPI)